Observations on The Activity of Acid and Alkaline Phosphatase in the Eggs, Nymphs and Adults of *Dysdercus cingulatus* (Fabr.) (Hemiptera: Pyrrhocoridae) in Relation to Development and Age

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IN
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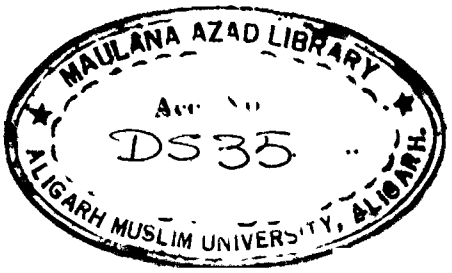
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CERTIFICATE

This is to certify that this piece of research work has been independently carried out by Mr. Mohd. Asif Khan. It is in the lines of modern research in Insect Physiology. I have permitted him to submit this dissertation for the partial fulfilment of the degree of Master of Philosophy in Zoology, Aligarh Muslim University, Aligarh.

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ABSTRACT

Phosphatases are the enzymes which play an important role in growth and reproduction of animals. The activity of acid and alkaline phosphatases has been studied by some workers in insect species but a little work has been done in hemipterous insects.

In the present research programme, the activity of acid and alkaline phosphatases has been studied in the eggs, nymphs and adults of Dysdercus cingulatus Fabr. in relation to their development and ageing. The phosphatase activity was determined by the method of Hawk et al. (1947).

In the freshly laid eggs the acid phosphatase activity was significantly higher than that of the alkaline phosphatase and it remained so during the course of embryonic development. Both the acid and alkaline phosphatases activity increased during the embryonic development but the rate of increase of alkaline phosphatase was comparatively higher than that of the acid phosphatase. However, the activity of both the enzymes declined after hatching.

In the 4th and 5th instar nymphs the acid phosphatase activity was more pronounced than the alkaline phosphatase activity throughout their development and growth. However, the

rate of increase of acid phosphatase activity was slower as compared to that of alkaline phosphatase activity. Both the enzymes exhibited maximum activity at the middle age of each nymphal instar and minimal prior to the moulting.

In newly emerged males the acid phosphatase activity was higher than that of newly emerged females. In females the acid phosphatase activity was maximum during the maturation of first batch of eggs. Following the oviposition it declined sharply and again the second peak appeared at the time of the maturation of second batch of eggs in the ovaries i.e. on the 11th post-emergent day. However, the second peak was lower than that of the first peak.

The alkaline phosphatase activity of males was similar to that of females when the maturation of oocytes was in its prime stage. However, at later stages of maturation of oocytes, the activity of alkaline phosphatases in female was always higher than that of males.

CONTENTS

I. INTRODUCTION

In the recent years insect physiologists have started concentrating on the characteristics and activity of enzymes (insect enzymology) involved in the biological activity of insects, because little is known in this field as compared to mammals. Among these enzymes Phosphatases are those enzymes, which catalyze the hydro lytic cleavage of phosphate esters. These are either acid or alkaline phosphatases. The former catalyze their substrates in the acidic medium, whereas the latter are active in the alkaline condition.

These enzymes play an important role on the growth and reproduction of animals, directly or indirectly. Further they are related to developmental processes, which include, tissue growth and tissue transformation, as well as excessive transport of materials. Since phosphatases regulate the transport of glucose, across the cell membrane, these enzymes are important in the sugar metabolism, during the growth and development of animals.

The characteristics, distribution and activity of acid and alkaline phosphatases have been studied, during the development and growth of many vertebrates by many workers, especially in fish (Al-Husaini, 1949; Roche, 1950; Kind and Mucchi, 1952; Needham, 1952; Krishnamoorthy, 1959; Prakash, 1961).

Among insects the distribution of acid and alkaline phosphatase as well as changes in its activity has been mostly studied through the developmental stages. This information has been fully reviewed in the next section. This information provides negligible data on the hemipterous species. Therefore, in the present investigation, the activity of acid and alkaline phosphatases has been biochemically studied in the developmental stages and adult Dysdercus cingulatus Fabr. which is one of the major pests of cotton in India.

II. REVIEW

Orthoptera.

Phosphatase activity of Orthopteriod insects was made by different workers. Fitzgerald (1949) determined the alkaline phosphatase activity in various stages of egg development of grasshopper Melanoplus differentialis. There was no detectable activity till the 12th day of development, it then increased until the onset of diapause, during which the activity increased slowly and then during post-diapause again increased rapidly.

Naqvi et al. (1967) quantitatively determined both acid and alkaline phosphatase activity in the desert locust Schistocerca gregaria. The pH optima of acid and alkaline phosphatase were found to vary in different stages indicating the effect of age. Srivastava and Saxena (1967) studied acid and alkaline phosphatases in the alimentary tract of different stages of Periplaneta americana. Alkaline phosphatase was widely distributed in the alimentary tract, the salivary glands, and the mal-phigian tubules of the nymphs and the adults. However, the acid phosphatase activity was absent in the newly hatched nymphs, whereas in adults positive activity occurred in the mid gut, the salivary glands and the malphigian tubules.

Vesuki and Dikshith (1968) observed the presence and activity of acid and alkaline phosphatases in the haemolymph, the testicular and the alimentary tissue of Poecilocerus pictus.

The haemolymph contained the maximum amount of alkaline and acid phosphatases. But the activity of these enzymes in the testicular tissue and the alimentary tract was weaker.

Naqvi and Ashrafi (1968) colorimetrically, measured acid and alkaline phosphatase activity in the developing eggs and the alimentary canal of aging nymphs as well as adult males and females of different ages of Schistocerca gregaria. Acid phosphatase activity increased with the embryonic development and was higher as compared to that of alkaline phosphatase. The alkaline phosphatase activity was lowest in the freshly laid eggs, but increased progressively as development proceeded. The activity of the enzymes was highest in the 1st-instar and gradually declined to the 5th-instar. In adults Schistocerca gregaria the activity of both the enzymes increased upto the maturation period and thereafter it gradually decreased. Acid phosphatase activity was higher in males, whereas alkaline phosphatase activity was generally higher in females.

Cook and Hipps (1969) determined phosphatases electrophoretically in the gastric secretion of cockroach, Periplaneta americana. They isolated three different phosphatases, one having an alkaline pH optimum near 7. Acid phosphatase was inhibited. The neutral phosphatase showed a marked increase in activity. In the reproductive organs of Schistocerca gregaria a histochemical study of alkaline phosphatase revealed its strong localisation in the nuclei of the spermathical epithelium (Qureshi, 1969).

Lauga (1972) observed clear activity of alkaline phosphatase in the haemolymph of larval stages of Acheta domesticus, while in that of imago it was only in feeble traces. Sinha and Mehrotra (1976) studied the property and distribution of alkaline phosphatase activity in various tissues of adult Schistocerca gregaria Forskal). The activity of this enzyme was higher in the fat body as compared to the nervous tissue and thoracic muscles.

Hemintera.

In this order histochemical localization of acid phosphatase has been studied in the ovary of Gerris remigis (Cone et al., 1966). Apart from this, there is no information on the changes in the activity of phosphatases on other species.

Coleoptera.

In the confused flour beetle, Tribolium confusum, Raychaudhuri et al.,(1965), studied phosphatases activity. Acid phosphatase activity in both the sexes, was greater than that of the alkaline phosphatase activity at any stage of adult life. The alkaline phosphatase activity in the female was found to be at a higher level than those in male, which maintained consistantly low level for a considerable length of adult life. But in neither sex, the activity fell to zero level at any stage of life. A relationship between the phosphatase activity and the capacity of the female to produce eggs throughout the adult life was found.

Further, in Tribolium confusum, Chaudhry et al., (1966), observed biochemical characteristics of alkaline phosphatase. Maximum phosphatase activity was obtained at 40°C. The intracellular localization of alkaline phosphatase showed that microsomes contained approximately one-fourth and mitochondria about one-tenth of the specific activity lodged in soluble fraction.

Trevel et al., (1970), studied the phosphorus metabolism and phosphatase activity during the metamorphosis of the meal worm, Tenebrio molitor. In the black carpet beetle, Attagenus megatoma, during larval development, the lowest and the maximum specific alkaline phosphatase activity for soluble fraction was recorded in the 2- and 9- mold larva respectively (Goginder and Linda, 1973).

Dhand and Rastogi (1975), studied the acid and alkaline phosphatase activity in relation to egg laying and aging in Callosobruchus analis. Acid phosphatase activity was much higher than that of alkaline phosphatase in both the sexes throughout adult life. Both the enzymes demonstrated two peaks of activity, first on the 3rd day and the second on the 10th day following emergence The first peak considered with egg laying activity in the female. Whereas the second peak for both enzymes was explained as responsible for metabolites, mobilising, before the death of insect.

Lepidoptera.

Sridhar and Bhat (1963), recorded the occurrence of both acid and alkaline phosphatases in the tissue extracts of intestine of Bombyx mori. The activity of both the enzymes progressively increased in each larval instar, and an abrupt decrease took place just before the next moult. Khatoon (1964) observed alkaline phosphatase activity in the mid gut of 5th instar larvae of Utethesia pulchella, with respect to age. In the newly moulted 5th instar larva the enzyme activity was stronger in the whole mid gut homogenate than those of mid gut tissue. Further, when the larvae were fed continiously following the moult, maximum activity occurred after 24 hours in homogenate of mid gut tissue. After it the enzyme activity declined in both homogenates.

In giant silk moth, Samia cynthia at least two acid phosphatases were present in the testicular homogenate, and the homogenate possessed higher activity (Gilbert, 1966). It decreased to 50% during development but increased to 75% of the pupal value before emergence of adult moth. Kageyama and Takashi (1974) studied the purification and properties of acid phosphatase. Two acid phosphatases were purified from the eggs of silk worm, Bombyx mori.

According to Kucera and Weiser (1974), in the infected last larval instar of Barathra brassicae, the activity of alkaline phosphatase rose above the normal activity two days after the last moult. The soluble acid phosphatases of the haemolymph and fat body of Calpodes ethlius (Stoll) indicated highest concentration in the fat body and it varied with stages of development (Collins, 1976). In the alimentary canal of the silk worm acid phosphatase first increased in the mid gut tissue and correspondingly in the mid gut content (Eguchi, 1976).

Diptera.

Barker and Alexander (1958) studied acid and alkaline phosphatase activity in separate homogenates of egg, larva, pupa and adult house fly, Musca domestica. During the entire length of life cycle, highest activity of alkaline phosphatase was recorded in 2 days old larva, and thereafter it decreased. Edward (1960) observed the post-emergence changes of enzyme activity in the mosquito Aedes aegypti. Acid phosphatase was maximum in one day old adult of both the sexes. Then it decreased to 28% by the end of 12 days in female and 30% after 18 days in male. The drop in phosphatase activity was thought due to change in enzyme molecules.

Changes in the activity of acid phosphatase were studied in different organs as well as in the whole fly homogenate (Pasteur et al., 1971). Similarly during the development of Drosophila pseudoobscura variation in acid phosphatase activity was also recorded by the same author. Both acid and alkaline phosphatases

increased notably at or near the middle of pupal life of face fly, Musca autumnalis, but there was a decrease in the activity of acid phosphatase during the first 4 days of adult life (Rousell and Georgia, 1971). It was followed by constant level recorded upto the end of the 3rd week, which was marked by a sharp drop. Subsequently, there was a low level of activity upto the end of 4th week. There was no significant difference in acid phosphatase activity between the sexes. Alkaline phosphatase activity increased during the 1st week and then gradually decreased during the remainder of adult life in both the sexes. The level of alkaline phosphatase activity in adult female was almost twice as high as in adult male.

The acid phosphatase activity in Drosophila melanogaster was pronounced in the eggs, but it was absent in the 1st and 2nd larval instars, again appeared in the 3rd instar and reached its peak in 24-28 hours after pupation (Mulharkar et al., 1972). It then decreased, at first gradually and abruptly reaching zero just before adult emergence.

Again in Drosophila melanogaster biochemical and genetic study of alkaline phosphatase was made by using Thyrosine-O-phosphate as a substrate (Harper et al., 1972). Terranova et al., (1973) made a study of soluble acid phosphatase from the posterior reproductive system of female house fly Musca domestica. In the beginning the enzyme activity was of zero order for 2 hours and it was temperature dependent. Disc electrophoresis showed that at least six protein had acid phosphatase activity and were pH dependent.

In the female house fly, Musca domestica, the acid phosphatase activity was histo-chemically studied and it was distributed throughout the vagina and associated structures (Leopold et al., 1973). However, the activity ranged from slight in the muscle tissue, moderate in the vaginal epithelium, to abundance in the secretory cells of accessory glands and spermetheca. Profuse amount of acid phosphatase activity associated with secretory tissues of spermetheca and accessory gland indicated that enzyme might be involved in the synthesis of materials necessary for sperm maintenance and fertilization.

In adult house fly, Musca domestica, the activity of acid phosphatase, a lysosomal marker in male adult greatly increased with age (Sohal and Maccarthy, 1973). Postlethwait and Gray (1975) studied the regulation of acid phosphatase activity in the ovary of Drosophila melanogaster. They observed that acid phosphatase activity increased approximately lograthmically for the first 2 days of adult life and then plateued at about 80 times, the level present at eclosion. The specific activity of acid phosphatase was constant for the first 15 hours, and then increased, by a factor 3 over the next 2 days. Analysis of staged follicles showed that the specific activity of acid phosphatase stands to increase at stage 10 days.

Hymenoptera.

In this order phosphatases have not been studied except in the honey bee Apis mellifica, in which Kubies (1971) studied isoenzymes of acid phosphatase by gel electrophoresis, using diazo coupling technique. Three main zones of activity appeared, each consisting of 2-5 electrophoretically distinct bands.

III. MATERIALS AND METHODS

1. Breeding and stock culture:

Dysdercus cingulatus Fabr. adults were collected from the cotton crop field and maintained in glass rearing jars measuring 8 x 4" in size. The bottom of these jars were filled with loose soil about 2" thick and the top of the jar was covered with a piece of muslin, which was tightly fixed by means of a rubber band. These rearing jars were kept at a temperature, 32° ± 1°C and 70-80% relative humidity. They were fed on soaked cotton seeds every day. The cotton seeds were soaked previously, over night. The females laid eggs in clusters on the moist soil. Then the adults were transferred to fresh jars. The eggs hatched after 4 or 5 days. Then the young nymphs were offered soaked, cotton seeds. From this nymphal stock, newly emerged adults were separated for further stock breeding.

2. Sampling of adults:

From the stock culture kept in rearing jars, freshly emerged adults were sorted out every day between 10 A.M. and 12.00 Noon. These adults were regarded to be of zero age. Each pair of such adult was placed in separate small rearing jars measuring 3 x 2" in size and was provided soaked cotton seeds

every day. In this way age-wise adult pairs were kept at the controlled temperature and humidity. Their maturing and oviposition were observed. After oviposition the eggs were separated and kept in the small jars and thus age-wise stock of the eggs was maintained. Age-wise maintenance of nymphs belonging to 4th and 5th instars also maintained by isolating newly moulted 4th and 5th instar nymphs from the stock culture. These stages were also fed on soaked cotton seeds and maintained at the above mentioned temperature and humidity.

3. Experimental procedure:

(a) Preparation of homogenate-

In the first series of experiment acid and alkaline phosphatase activity was determined in the eggs, freshly laid and belonging to different age upto their hatching. For this purpose few eggs of each age were weighed and homogenized in 1.0 ml. of distilled water in homogenising tubes.

In the second series of experiment phosphatase activity was studied in the whole body of the nymphs of different age belonging to 4th and 5th instars. For this purpose nymphs of each age were weighed and homogenised in 1.0 ml. distilled water separately. In the third series of experiment the phosphatase activity was observed in the whole body homogenate of male and female from the time of their emergence to 11 day old adults.

In this case, individual adult of each sex was weighed and then homogenised in 1.0 ml. distilled water. At a time homogenate of two members of each sex of different age were prepared. All the homogenates were centrifuged at 4,000 r.p.m. for 15 minutes. The supernatants were used for the determination of phosphatases activity.

(b) Biochemical procedure -

The technique for determination of acid and alkaline phosphatases activity in the homogenates was quantitative and based on the modification of the Colorimetric method of Hawk, Oser and Summerson (1947). The detailed procedure is given below. For determining the activity of acid and alkaline phosphatases, acid and alkaline phosphate substrates (Sodium B-glycerophosphate - Sodium diethyl barbiturate kept at 38°C) were used respectively. The buffer solutions of acid and alkaline phosphate substrate were adjusted to 5.4 and 8.7 respectively by adding acid or alkali as necessary. From each homogenate three samples were used as experimental (incubated), control (un-incubated) and reagent blank. Thus from each homogenate two aliquot each of 0.1 ml. were drawn. One of these samples which received 9.0 ml substrate (acid/alkaline) mixed, then kept for one hour incubation at 37°C. After which it was cooled in ice cold bath for 15 minutes. Then 2.0 ml. of 30% Trichloro acetic acid was mixed. After 5 minutes it was filtered through filter paper (Whatman 42). In the respective

sample, 9.0 ml. of substrate (acid/alkaline) and 2.0 ml. of 30% Trichloro-acetic acid was mixed and then filtered through filter paper (Whatman 42). Finally, 9.0 ml. of each filtrate was given 1.0 ml Molybdate II reagent, 0.4 ml. Amino-nephthol-sulphonic acid reagent and 0.6 ml. distilled water to make up the volume 10.0 ml. in each final solution. After mixing, let the samples stand for 5 minutes. The colour was developed. Then the colour density of both the final solutions were compared with a reagent blank containing 8.0 ml. of 5% Trichloro-acetic acid, 1.0 ml. of Molybdate II, 0.4 ml. of Amino-nephthol-sulphonic acid reagent and 0.6 ml. of distilled water. The colour density of both the samples were recorded by Erma photoelectric Colorimeter using red filter ranging 660. The colour density depends on the amount of phosphorus released. To appreciate the difference in colour density of various final solutions reliable dilution curve was plotted (Figures 1, 2 for acid and alkaline phosphatase activity respectively) by using standard phosphate solution containing monopotassium phosphate. This solution contained 0.04 mg. of phosphorus in 8.0 ml. of 5% T.C.A. The detailed procedure is given in Tables(1 and 2.).

The differences between the colour density of experimental and their respective control samples gave the phosphatase activity. Therefore, the total inorganic phosphorus released

in known weight of eggs, nymphs and adult body was known. Finally, the inorganic phosphorus per mg. of tissue weight was calculated. Thus, the phosphatase activity was expressed in terms of inorganic phosphorus released per mg. of body weight. A number of replication for each age group was made and the entire data are tabled in the appendix tables. The arithematic mean was calculated for each observation and standard deviation was calculated by the following formula :

$$S.D. = \sqrt{\frac{\varepsilon d^2}{N-1}}$$

where εd^2 = Sum of square of the difference of mean value.

N = No. of observations.

TABLE 1.

Showing data for the dilution curve.

(For acid phosphatase activity)

Treatments	Serial dilutions								
	Blank	1	2	3	4	5	6	7	8
Quantity of standard solution (ml.)	-	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0
Ammonium molybdate added (ml.)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Amino nephthol sulphonic acid reagent (ml.)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Quantity of water added to make up volume 10 ml. (ml.)	8.6	7.6	6.6	5.6	4.6	3.6	2.6	1.6	0.6
Optical density	-	0.081	0.164	0.244	0.328	0.409	0.482	0.585	0.638
Phosphorus concentration (mg.)	-	0.005	0.01	0.015	0.02	0.025	0.03	0.035	0.04

Strength of solution = 8 ml/0.04 mg. of phosphorus.

TABLE 2.

Showing data for the dilution curve
(for Alkaline phosphatase activity)

Treatments	Serial dilutions								
	Blank	1	2	3	4	5	6	7	8
Quantity of standard solution (ml.)	-	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0
Ammonium molybdate added (ml.)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Amino nephthol sulphonic acid reagent (ml.)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Quantity of water added to make up volume 10 ml. (ml.)	8.6	7.6	6.6	5.6	4.6	3.6	2.6	1.6	0.6
Optical density	-	0.111	0.131	0.272	0.352	0.456	0.502	0.602	0.699
Phosphorus concentration (mg.)	-	0.005	0.01	0.015	0.02	0.025	0.03	0.035	0.04

Strength of solution = 8 ml./0.04 mg of phosphorus.

Fig. 1. Dilution curve for acid phosphatase activity.

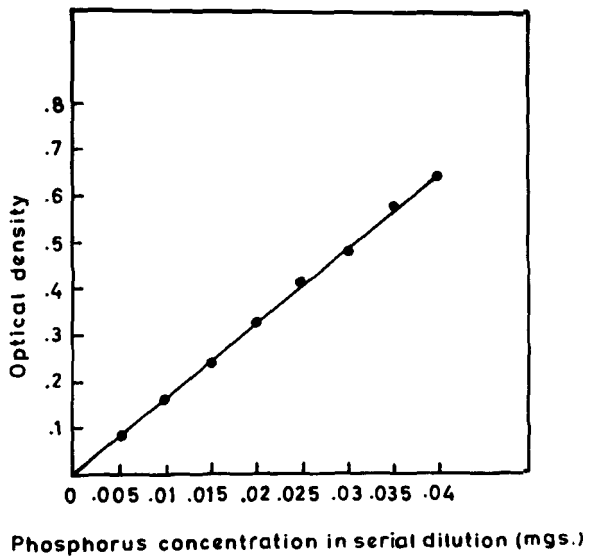

Optical density
.8
.7
.6
.5
.4
.3
.2
.1
0 .005 .01 .015 .02 .025 .03 .035 .04
Phosphorus concentration in serial dilution (mgs.)

Fig. 2. Dilution curve for alkaline phosphatase activity.

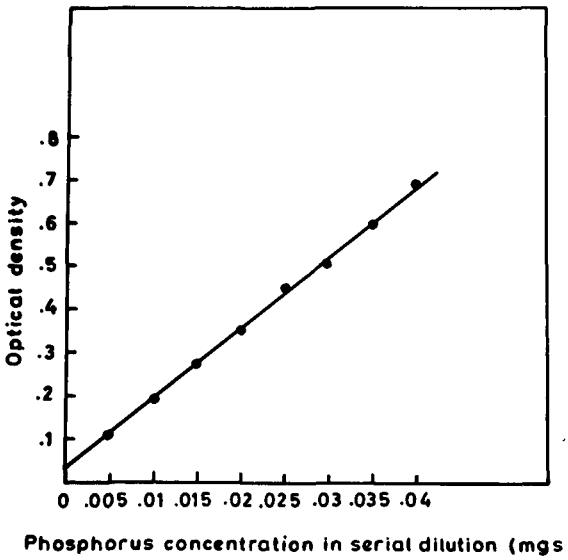
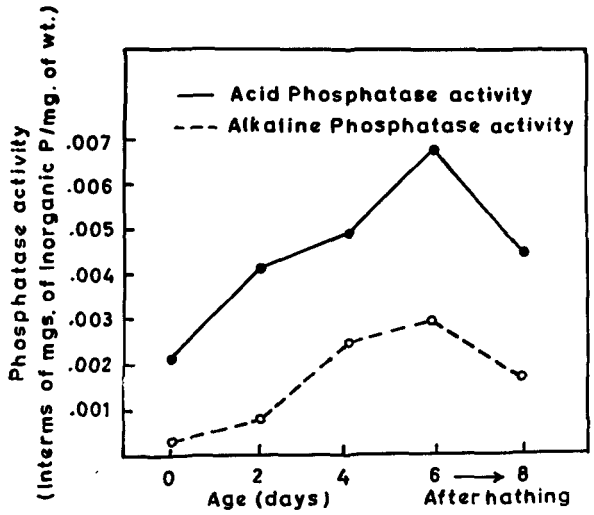

Optical density
.8
.7
.6
.5
.4
.3
.2
.1
0 .005 .01 .015 .02 .025 .03 .035 .04
Phosphorus concentration in serial dilution (mgs

Fig. 3. Showing acid and alkaline phosphatase activity during the embryonic development in eggs of *P. cingulatus*.


Acid Phosphatase activity
Alkaline Phosphatase activity
Phosphatase activity
(Interms of mgs. of Inorganic P/mg. of wt.)
.007
.006
.005
.004
.003
.002
.001
0
2
4
6 → 8
Age (days)
After hathing

increased throughout during the embryonic development and until eclosion took place on the 6th day following oviposition. However, the rate of increase was slow in comparison to that occurring between 2nd and 4th day (Figure 3).

Further, the newly hatched nymphs had weaker activity of this enzyme. Throughout the embryonic development, the acid phosphatases activity was higher than that of alkaline phosphatase activity. But the rate of increase of alkaline phosphatase activity was more pronounced than that of acid phosphatase activity during the early embryonic development. Maximum activity of alkaline phosphatase yielded was 0.00302 mg. Pi/mg. egg weight (Table 3). As compared to the maximal activity of acid phosphatases than that of alkaline phosphatases was less than half of the former (Figure 3).

Table 3 Showing phosphatases activity during embryonic development of eggs of *D. circulatus*.

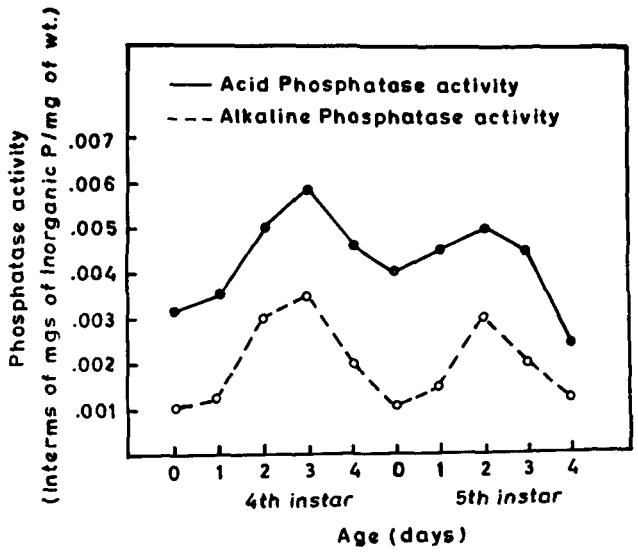
Sl. No.	Age (days)	Acid phosphatase activity: Mean of inorganic phosphorus released/ mg. weight of eggs (mg.)	S.D.	Alkaline phosphatase activity: Mean of inorganic phosphorus released/ mg. weight of eggs. (mg.)	S.D.
1.	0	0.00214	± 0.000318	0.000307	± 0.000062
2.	2	0.00415	± 0.000248	0.000781	± 0.000045
3.	4	0.00491	± 0.000133	0.00250	± 0.000044
4.	6	0.00577	± 0.000212	0.00302	± 0.000265
	After hatching				
5.	8	0.00455	± 0.000107	0.00176	± 0.0000798

V. Phosphatase activity in the whole body of 4th and 5th instar hoppers of Dysdercus cingulatus.

In the next series of experiments the activity of acid and alkaline phosphatases was determined in the 4th and 5th nymphal instars. In the whole body homogenate of the newly moulted 4th instar nymphs, the acid phosphatases activity was more pronounced than that of the alkaline phosphatases. Acid phosphatases activity was three times more than that of alkaline phosphatase activity at this stage i.e. 0.00316 and 0.001 mg Pi/mg. of body weight respectively, (Table 4). Thereafter the activity of both types of enzymes increased as the growth proceeded.

However, the rate of increase of the acid phosphatases activity was slower as compared to that of alkaline phosphatase activity. The acid phosphatases activity attains the highest activity on the 3rd day following moulting from the 3rd instar nymph (Appendix Table 9) and at this stage the average activity recorded was 0.00591 mg Pi/mg of body weight (Table 4). The alkaline phosphatase also showed maximal activity in the 4th instar nymphs of the same age when acid phosphatases activity was maximum. But the alkaline phosphatases activity was much weaker than that of the acid phosphatases and then maximum activity of the former enzyme was 0.00353 mg. Pi/mg. of body weight.

Fig. 4. Showing acid and alkaline phosphatase activity of 4th and 5th instars of *D. cingulatus* related with age and moulting.


Acid Phosphatase activity
Alkaline Phosphatase activity
Phosphatase activity
(Interms of mgs of Inorganic P/mg of wt.)
.007
.006
.005
.004
.003
.002
.001
0 1 2 3 4 0 1 2 3 4
4th instar
5th instar
Age (days)

After the 3rd day the activity of both the enzymes start decreasing and thus soon after the next moulting (zero age of 5th instar) the value of acid phosphatase activity becomes 0.004 mg. Pi/mg. of body weight which is however remarkably more than that of the newly moulted (zero age) 4th instar nymphs. But as compared to that of this enzyme the activity of the alkaline phosphatases drops more remarkably and reaches upto the level of the zero age 4th instar nymph (Figure 4). In the newly moulted 5th instar nymph thereafter the phosphatases activity of both enzyme commenced rising as the development proceeds. In this instar also there was a peak of activity of both the enzymes in two day old nymphs.

However, these peaks are correspondingly lower than those of the 4th instar (Figure 4). The maximal activity of acid phosphatases was 0.00499 mg of Pi/mg. of body weight whereas that of alkaline phosphatases was 0.00298 mg of Pi/mg. of body weight. Thereafter, both acid and alkaline phosphatases activity started decreasing till the next moulting. Throughout the growth period of the 5th instar, the acid phosphatases activity was always higher than that of the alkaline phosphatase activity.

Table 4 Showing phosphatases activity in the 4th- and 5th instar of D. *cingulatus*.

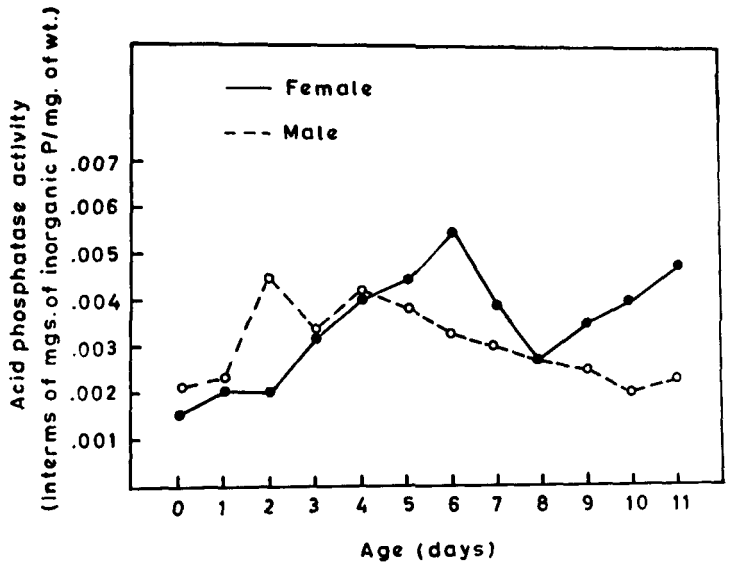
Age (days)	Acid phosphatase activity: Mean of inorganic phosphorus released/ mg. of body weight (mg.)	S.D.	Alkaline phosphatase activity: Mean of inorganic phosphorus released/ mg. of body weight (mg.)	S.D.
4th-instar				
0	0.00316	± 0.00018	0.000979	± 0.0000707
1	0.00349	± 0.00019	0.00117	± 0.0000447
2	0.00501	± 0.00018	0.00297	± 0.000146
3	0.00591	± 0.00024	0.00353	± 0.000131
4	0.00458	± 0.00029	0.00204	± 0.0000742
5th-instar				
0	0.00400	± 0.0000937	0.00101	± 0.0000345
1	0.00449	± 0.0000731	0.00149	± 0.0000415
2	0.00499	± 0.000149	0.00298	± 0.0000674
3	0.00447	± 0.0000760	0.00202	± 0.0000436
4	0.00240	± 0.000062	0.00121	± 0.0000298

VI, Phosphatase activity in Adult Dysdercus cingulatus related with age and reproduction.

Phosphatase activity was determined in the adults male and female from the time of emergence (zero age) to eleven days. In the newly emerged (zero age) males, the acid phosphatases activity was recorded 0.00216 mg. of Pi/mg. of the body weight. It initially increased slowly, within the next 24 hours. But later there was a rapid rise reaching to its peak level in 2 day old males (Figure 5). Although, there was a steep fall during the next 24 hours. The average value of activity in 4 day old male was not much different than that of the 2 day old males (Table 5). However, the maximum acid phosphatase activity was 0.00450 mg. of Pi/mg. body weight, whereas the next higher value was 0.00419 mg. of Pi/mg. of body weight. Following this there was a gradual decrease of the activity and finally on the 11 day it reached 0.00231 mg. of Pi/mg. of body weight, which was slightly higher than the initial value in the newly emerged adults (Table 5).

In the newly emerged females the acid phosphatase activity was 0.00151 mg. of Pi/mg. of body weight which was lower than that of the newly emerged males (Figure 5). Further, in contrast to that of males, the activity of this enzyme enhanced progressively although initially within the first two

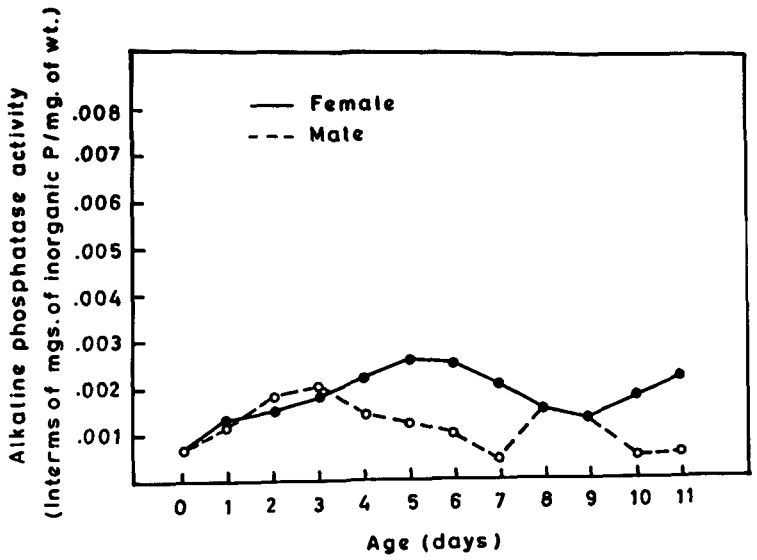
Fig. 5. Showing acid phosphatase activity in adult *D. cingulatus* related with age.


Female
Male
Acid phosphatase activity
(Interms of mgs. of inorganic P/mg. of wt.)
.007
.006
.005
.004
.003
.002
.001
0 1 2 3 4 5 6 7 8 9 10 11
Age (days)

days the rate of increasing the activity was slow. Thus, in the 6 day old females there was maximum activity i.e. 0.00548 mg. of Pi/mg. of body weight which was about four times more than that of the newly emerged females (Table 5). It was followed by a decrease during the next two days when the value of the activity reached to a level of 0.00276 mg. Pi/mg. of body weight in 8 day old female. Again the enzyme showed a sharp increase in its activity and a second peak (0.00478 mg. Pi/mg. of body weight) was present on the 11th post emergent day. Generally in the females acid phosphatase activity was higher than that of the males after the 3rd post-emergent day and further, it had two pronounced peaks, although the second peak was slightly lower than that of first.

In the newly emerged males the alkaline phosphatases activity was 0.000679 mg. Pi/mg. of body weight. It then gradually rose as the age advanced and thus in the 3 day old males the activity was 0.00206 mg. Pi/mg. of body weight. However, afterwards the alkaline phosphatase activity of the older males declined. Consequently in seven day old males the activity fell to an extent lower to that of newly emerged males (Table 6). In the seven day old males the activity was 0.000415 mg. Pi/mg. of body weight which was the lowest ever during the 11 day period. It was again followed by a sharp increase which developed the second peak of activity, when the activity reached to 0.00154 mg. Pi/mg. of body weight. This

Fig. 6. Showing alkaline phosphatase activity in adult *D. cingulatus* related with age.


Alkaline phosphatase activity
(Interms of mgs. of inorganic P/mg. of wt.)
.008
.007
.006
.005
.004
.003
.002
.001
Female
Male
0 1 2 3 4 5 6 7 8 9 10 11
Age (days)

value was lower than that of the first peak. During this period the rate of increase was much pronounced. Again, the activity of alkaline phosphatase started decreasing in subsequently older males and thus 11 day old males had 0.000588 mg. Pi/mg. of body weight which was almost equal to the value observed in 7 day old males (Table 6).

In the newly emerged females the alkaline phosphatase activity was similar to that of freshly emerged males. But, later, in the females of subsequent age the alkaline phosphatase activity progressively increased. The maximal activity was found in 5 day old females, as against 3 day old males (Figure 6). Further, this value of maximal activity in female Dysdercus cingulatus was 0.00258 mg. Pi/mg. of body weight as against 0.00206 mg. Pi/mg. of body weight. However, the females later, showed slow decrease in alkaline phosphatases activity as compared to that of males. Although 9 day old females had lowest value after the peak value; it was remarkably higher than that of the newly emerged females.

During the 8th and 9th post-emergent days the alkaline phosphatase activity of the males and the females was similar. But in the females, alkaline phosphatase activity further developed progressively and thus 11 day old females had 0.00218 mg. Pi/mg. of body weight which was much appreciably higher than that of the males of the corresponding age (Figure 6).

In general it can be inferred that following emergence females had higher activity of alkaline phosphatases than that of males (Table 6).

TABLE 5. Showing acid-phosphatase activity in male and female *D. circulatum* in relation to their age.

Age (days)	Males		Females	
	Mean of inorganic phosphorus released/ mg. of body weight (mg.)	S.D.	Mean of inorganic phosphorus released/ mg. of body weight (mg.)	S.D.
0	0.00216	± 0.000148	0.00151	± 0.0000605
1	0.00235	± 0.000123	0.00199	± 0.000121
2	0.00450	± 0.000149	0.00205	± 0.000102
3	0.00331	± 0.000155	0.00314	± 0.000134
4	0.00419	± 0.000260	0.00399	± 0.000484
5	0.00381	± 0.000184	0.00439	± 0.000148
6	0.00336	± 0.000160	0.00548	± 0.000215
7	0.00303	± 0.000148	0.00382	± 0.000397
8	0.00274	± 0.000180	0.00276	± 0.000179
9	0.00264	± 0.0000815	0.00349	± 0.0000115
10	0.00201	± 0.000182	0.00399	± 0.000912
11	0.00231	± 0.000126	0.00478	± 0.000116

Table 6 Showing alkaline-phosphatase activity in male and female *D. cingulatus* in relation to their age.

Age (days)	Males		Females	
	Mean of inorganic phosphorus released/ mg. of body weight (mg.)	S.D.	Mean of inorganic phosphorus released/ mg of body weight (mg.)	S.D.
0	0.000679	± 0.000059	0.000722	± 0.0000743
1	0.00119	± 0.000124	0.000846	± 0.0000952
2	0.00178	± 0.0000615	0.00150	± 0.0000799
3	0.00206	± 0.00013	0.00177	± 0.0000699
4	0.00141	± 0.000134	0.00219	± 0.0000700
5	0.00123	± 0.0000709	0.00258	± 0.0000899
6	0.00107	± 0.000132	0.00249	± 0.0000993
7	0.000415	± 0.000531	0.00197	± 0.000186
8	0.00154	± 0.000113	0.00149	± 0.000484
9	0.00137	± 0.0000416	0.00128	± 0.0000609
10	0.000527	± 0.000066	0.00178	± 0.0000240
11	0.000528	± 0.0000804	0.00218	± 0.0000576

VII. DISCUSSION

In the present observations in *Dysdercus cingulatus*, the occurrence of the acid phosphatases activity more than that of the alkaline phosphatases suggests the predominent synthesis of the former enzyme in the freshly laid eggs. The activity of the acid phosphatases was also recorded in the eggs of house flies, *Musca domestica*, (Barker and Alexander, 1958), stable fly, *Stomoxys calcitrans* (Ashrafi and Fisk, 1961), *Drosophila melanogaster* (Mulherker *et al.*, 1972). It is further evident that throughout the development in the eggs of *D. cingulatus*, there is progressive increase in the synthesis of the acid phosphatases. Thus the activity of the acid phosphatases in the developing eggs of *D. cingulatus* is like that of the eggs of the silk worm *Bombyxmori* (Sridhar and Bhat, 1963) and *Schistocerca gregaria* (Naqvi *et al.*, 1968).

The higher synthesis of the acid phosphatases during the progressive tissue formation and growth within the eggs is obviously related to the increasing metabolism and transport of the substances especially the glucose. However, the con. ..tra tion of the alkaline phosphatases is weaker than that of the acid phosphatases, but the former rapidly increases in the

beginning of the development. Such condition was also recorded in the developing eggs of grass hopper Schistocerca gregaria (Fitzgerald, 1949), although in these eggs the activity of alkaline phosphatase was absent for a day following oviposition. In the eggs of Bombyx mori also there is lack of alkaline phosphatases activity in the early stages of embryonic development (Sridhar and Bhat, 1963). But Barker and Alexander (1956) in the eggs of house fly Musca domestica reported high activity of the alkaline phosphatases, a condition which occurs in the eggs of Dysdercus cingulatus after some days following oviposition. when embryonic development is in progress. Thus it appears that in the eggs of house fly, the activity of the alkaline phosphatases was determined only in the old eggs and not in the freshly laid eggs.

During the 4th and 5th instars of Dysdercus cingulatus, the activity of both acid and alkaline phosphatases followed similar trend of changes. These enzymes had maximum activity in the middle aged nymphs in each instar. Such a phenomenon appears to be related with the metabolic changes during the growth and moulting. The progressive increase of the phosphatases activity during the first half of each instar may be related with the active metabolism of substances required for laying down the new cuticle before the next moult. Since phosphatases are concerned with the release of inorganic phosphorus which is utilized in the transport of glucose and

other reducing sugars, their concentration during the active metabolism must go up in the body for the synthesis of polysaccharides which are utilised in the formation of the new cuticle as well as for building the body tissue. Thus the synthesis and activity of the phosphatases in the body of the nymphs of Dysdercus cingulatus appears to be related with the hormonal control during the growth and moulting. In this regard, the moulting hormone (ecdysone) may be involved in regulating the synthesis and secretion of the phosphatases as well.

It is well known that during the growth of the larval forms moulting hormone initially stimulates the activity of the epidermal cells of the integument, oenocytes and the hypodermal glands which are subjected to increased synthesis of secretory material for laying down the new cuticle (Wigglesworth, 1973). Once the new cuticle is under the process of being laid, the enzymes involved in the process are gradually less synthesized. There is progressively enhancement of concentration of the total proteins of the body in the 4th and 5th instars of Dysdercus cingulatus during the first half of each instar and gradual decrease prior to next moulting (Shamim Sifat 1977). Such data are also parallel with that of phosphatases activity in this species as previously recorded.

Thus, the activity of the phosphatases is one of the facets of the metabolic activity in the body during the larval growth and moulting which must run parallel with other metabolic changes. In the last larval instar of Dysdercus cingulatus also there was a maximal activity of alkaline phosphatase in the middle aged larvae and prior to pupation, this activity progressively decreased (Khatoon, 1964)

In female Dysdercus cingulatus, the acid phosphatases activity progressively rises after emergence and it is related with the maturation of the ovaries and thus prior to oviposition of the first batch of eggs by the females, the activity of the acid phosphatases was maximum. It was lower following oviposition. Thus the synthesis of this phosphatase in the females is related to the metabolic function for the deposition of yolk. This view can be further supported by the fact that before the oviposition of the second batch of eggs, there is again increase in the activity of acid phosphatases. It is therefore inferred that this enzyme in female Dysdercus cingulatus undergoes cyclic changes related with the yolk deposition in the eggs. Such a cyclic activity of this enzyme is absent in the males. It is further interesting that the changes in the total proteins of the whole body as well as that of ovaries of Dysdercus cingulatus (Sifat and Khan, 1974) correspond to that of the activity of this enzyme. In female Callosobruchus analis also cyclic changes in acid phosphatase activity was observed (Dhand and Rastogi, 1976).

Similarly, Raychaudhri and Buts (1965b) also reported two peaks of acid phosphatases activity in Tribolium confusum females. However, in Tenebrio molitor (Ludwig et al. 1962), Schistocerca gregaria (Naqvi et al., 1968), Musca autumnalis (Rousell, 1971) recorded only peak in early life.

Naqvi et al. (1968) also recorded high acid phosphatases activity in the young females whereas aging was related with decreasing activity. Thus, in these species there appears to be no cyclic change in the activity of acid phosphatases related with maturation. In this regard Dysdercus cingulatus differs from these species.

It is further evident that in the male Dysdercus cingulatus, the activity of the acid phosphatases does not change cyclically and in them the activity rises to some extent in the early adult life, then gradually goes down with aging. In this respect, therefore, the male Dysdercus cingulatus resemble the Tenebrio molitor, Schistocerca gregaria and Musca domestica.

In the eggs, the nymphs and the adults of Dysdercus cingulatus, the activity of the acid phosphatases generally remained higher than that of the alkaline phosphatases. It may be concluded that the synthesis of acid phosphatases throughout the life-history of this species dominates over that of the alkaline phosphatases and this is so because the tissues of this insect may provide more favourable condition for the activity of the former enzymes to mobilise the metabolites for tissue building.

VIII. SUMMARY

1. In *Dysdercus cingulatus* Fabr. (Hemiptera: Pyrrhocoridae) acid and alkaline phosphatases activity was studied in the homogenates of eggs, 4th and 5th instar nymphs and adults of both sexes in relation to their development and reproduction.

2. In the eggs, the acid phosphatase activity was always higher than that of the alkaline phosphatase activity during the embryonic development.

3. The activity of both the enzymes declined sharply after hatching.

4. In the 4th and 5th instar nymphs the activity of acid phosphatases was higher than that of alkaline phosphatase during the moulting and their development.

5. The activity of both the enzymes declined gradually during the intermoulting period.

6. The activity of both the enzymes in 4th instar nymphs was slightly higher than that of the 5th instar nymphs.

7. In freshly emerged females the acid phosphatase activity was slightly lower than that of the males of the corresponding age but later it increased sharply.

8. The increase in the acid phosphatases activity in females was in relation to the development and maturation of eggs.

9. In females the acid phosphatases activity was always higher than alkaline phosphatase activity.

10. The alkaline phosphatase activity of males was similar to that of females when the maturation of oocytes was in its prime stage. However, at later stages of maturation of oocytes it was always higher in females than that of males of the corresponding age.

11. Acid and alkaline phosphatase activity was related to tissue growth, maturation and metabolism directly or indirectly.

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* Original not available.

APPENDIX TABLE 1. Zero day old eggs

No. of samples	Wt. of eggs (mg)	Acid phosphatase activity					Alkaline phosphatase activity				
		Optical density of		Pi in the		Pi per mg. wt. of eggs (mg)	Optical density of		Pi in the		Pi per mg. wt. of eggs (mg)
		Exp.	Control	aliquot (mg)	whole eggs (mg)		Exp.	Control	aliquot (mg)	Whole eggs (mg)	
1.	70.0	0.409	0.181	0.014	0.14	0.00200	0.824	0.796	0.0025	0.025	0.000385
2.	50.0	0.332	0.161	0.01	0.10	0.00200	0.403	0.161	0.0014	0.014	0.000280
3.	55.0	0.409	0.211	0.012	0.12	0.00218	0.661	0.332	0.00145	0.0145	0.000254
4.	53.0	0.538	0.362	0.011	0.11	0.00207	0.488	0.252	0.0014	0.014	0.000264
5.	39.0	0.240	0.111	0.008	0.08	0.00205	0.292	0.280	0.0011	0.011	0.000282
6.	45.0	0.594	0.444	0.009	0.09	0.00200	0.362	0.332	0.002	0.02	0.000444
7.	43.0	0.362	0.222	0.0085	0.085	0.00197	0.211	0.191	0.0012	0.012	0.000279
8.	65.0	0.569	0.256	0.019	0.019	0.00292	0.292	0.260	0.00187	0.0187	0.000276
Mean		52.50				0.00214					0.000307
S.D.		±10.71				±0.000318					±0.0000682

Note: Aliquot taken = 0.1 ml.
Water added = 0.9 ml.

APPENDIX TABLE 2. Two day old eggs

No. of samples	Wt. of eggs (mg)	Acid phosphatase activity: Optical density of Exp.	Acid phosphatase activity: Optical density of Control	Acid phosphatase activity: Pi in the aliquot (mg)	Acid phosphatase activity: Pi in the whole eggs (mg)	Acid phosphatase activity: Pi per mg. wt. of eggs (mg)	Alkaline phosphatase activity: Optical density of Exp.	Alkaline phosphatase activity: Optical density of Control	Alkaline phosphatase activity: Pi in the aliquot (mg)	Alkaline phosphatase activity: Pi in the whole eggs (mg)	Alkaline phosphatase activity: Pi per mg. wt. of eggs (mg)
1.	48.0	0.523	0.201	0.02	0.20	0.00416	0.299	0.222	0.004	0.04	0.000833
2.	60.0	0.523	0.111	0.0255	0.255	0.00425	0.398	0.314	0.005	0.05	0.000757
3.	35.0	0.444	0.211	0.014	0.140	0.00400	0.362	0.323	0.0025	0.025	0.000714
4.	32.00	0.444	0.244	0.012	0.120	0.00390	0.252	0.222	0.0025	0.025	00000781
5.	30.0	0.545	0.328	0.0135	0.135	0.00450	0.248	0.211	0.0024	0.024	0.000833
6.	48.0	0.548	0.362	0.0185	0.185	0.00385	0.292	0.244	0.0035	0.035	0.000729
7.	51.0	0.561	0.181	0.023	0.230	0.00450	0.420	0.362	0.004	0.04	0.000784
8.	61.0	0.502	0.111	0.0255	0.255	0.00409	0.398	0.323	0.005	0.05	0.000819
Mean		45.62				0.00415					0.00781
S.D.		±12.10				± 0.000248					± 0.0000457

Note: Aliquot taken = 0.1 ml.
Water added = 0.9 ml.

APPENDIX TABLE 3. Four day old eggs

No. of samples	wt. of eggs (mg)	Acid phosphatase activity					Alkaline phosphatase activity				
		Optical density of		Pi in the		Pi per mg wt. of eggs (mg)	Optical density of		Pi in the		Pi per mg wt. of eggs (mg)
		Exp.	Control	aliquot (mg)	whole eggs (mg)		Exp.	Control	aliquot (mg)	whole eggs (mg)	
1.	34.0	0.482	0.201	0.0165	0.165	0.00485	0.301	0.171	0.0085	0.085	0.00280
2.	51.0	0.577	0.174	0.0245	0.245	0.00480	0.444	0.233	0.0125	0.125	0.00245
3.	49.0	0.611	0.191	0.025	0.25	0.00510	0.462	0.255	0.01255	0.1255	0.00255
4.	53.0	0.585	0.161	0.026	0.26	0.00490	0.509	0.284	0.0135	0.135	0.00254
5.	38.0	0.569	0.260	0.019	0.19	0.00500	0.409	0.252	0.0095	0.095	0.00250
6.	42.0	0.602	0.280	0.021	0.21	0.00500	0.319	0.155	0.01075	0.1075	0.00250
7.	49.0	0.602	0.215	0.023	0.23	0.00469	0.392	0.191	0.012	0.12	0.00244
8.	37.0	0.594	0.282	0.0185	0.185	0.00500	0.462	0.305	0.0095	0.095	0.00256
Mean		44.12				0.00491					0.00250
S.D.		±7.259				±0.000133					±0.000044

Aliquot taken = 0.1 ml.
Water added = 0.9 ml.

APPENDIX TABLE 4. Six day old eggs

No. of samples	Wt. of eggs (mg)	Acid phosphatase activity					Alkaline phosphatase activity				
		Optical density of		Pi in the		Pi per mg wt. of eggs (mg)	Optical density of		Pi in the		Pi per mg. weight of eggs (mg)
		Exp.	Control	aliquot (mg)	whole eggs (mg)		Exp.	Control	aliquot (mg)	whole eggs (mg)	
1.	32.0	0.561	0.208	0.021	0.21	0.00651	0.444	0.292	0.009	0.09	0.00281
2.	38.0	0.602	0.161	0.0265	0.265	0.00697	0.462	0.264	0.012	0.12	0.00315
3.	35.0	0.611	0.211	0.024	0.24	0.00685	0.482	0.310	0.01	0.1	0.00285
4.	42.0	0.561	0.111	0.027	0.27	0.00642	0.292	0.108	0.012	0.12	0.00285
5.	49.0	1.022	0.233	0.034	0.34	0.00693	0.523	0.284	0.015	0.15	0.00306
6.	50.0	0.824	0.252	0.035	0.35	0.00700	0.432	0.181	0.0154	0.154	0.00300
7.	44.0	0.854	0.240	0.03	0.30	0.00681	0.569	0.342	0.0145	0.145	0.00362
8.	40.0	0.658	0.211	0.027	0.27	0.00675	0.561	0.382	0.0116	0.116	0.0029
Mean		41.75				0.00677					0.00302
S.D.		± 6.341				± 0.000212					± 0.000265

Aliquot taken = 0.1 ml.
Water added = 0.9 ml.

APPENDIX TABLE 5. Hatchlings

No. of samples	Wt. of hatchings	Acid phosphatase activity: Optical density of Exp.	Acid phosphatase activity: Optical density of Control	Acid phosphatase activity: Pi in the aliquot (mg)	Acid phosphatase activity: Pi in the Hatchlings (mg)	Acid phosphatase activity: Pi per mg. hatchling body weight(mg)	Alkaline phosphatase activity: Optical density of Exp.	Alkaline phosphatase activity: Optical density of Control	Alkaline phosphatase activity: Pi in the aliquot (mg)	Alkaline phosphatase activity: Pi in the Hatchlings (mg)	Alkaline phosphatase activity: Pi per mg. hatchling body weight(mg)
1.	38.0	0.488	0.211	0.017	0. 17	0.00447	0.357	0.264	0.0065	0.065	0.00171
2.	28.0	0.495	0.284	0.013	0. 13	0.00464	0.280	0.204	0.0050	0.05	0.00185
3.	39.0	0.509	0.233	0.0175	0.175	0.00448	0.372	0.272	0.0065	0.065	0.00166
4.	44.0	0.516	0.208	0.0195	0.195	0.00443	0.392	0.204	0.008	0.08	0.00181
5.	43.0	0.577	0.244	0.02	0. 20	0.00465	0.392	0.194	0.008	0.08	0.00186
6.	54.0	0.569	0.204	0.023	0. 23	0.00444	0.328	0.184	0.009	0.09	0.00166
7.	50.0	0.611	0.233	0.023	0. 23	0.00460	0.372	0.233	0.009	0.09	0.00180
8.	51.0	0.569	0.181	0.024	0. 24	0.00470	0.426	0.292	0.009	0.09	0.00176
Mean		43.37				0.00455					0.00176
S.D.		± 8.450				± 0.000107					± 0.0000798

Note: Aliquot taken = 0.1 ml.
Water added = 0.9 ml.

APPENDIX TABLE 6. 4th Instar Zero-day-old.

No. of sample.	wt. of body (mg)	Acid phosphatase activity					Alkaline phosphatase activity				
		Optical density of		Pi in the		Pi per mg. wt. of body (mg)	Optical density of		Pi in the		Pi per mg. wt. of body (mg)
		Exp.	Control	aliquot (mg)	whole body (mg)		Exp.	Control	aliquot (mg)	whole body (mg)	
1.	33.5	0.414	0.252	0.01	0.1	0.00298	0.292	0.252	0.003	0.03	0.000895
2.	30.0	0.432	0.260	0.0105	0.105	0.00350	0.252	0.194	0.003	0.03	0.000999
3.	34.0	0.420	0.268	0.0105	0.105	0.00308	0.332	0.284	0.0035	0.035	0.00102
4.	37.0	0.469	0.280	0.012	0.12	0.00324	0.314	0.252	0.004	0.04	0.00108
5.	35.0	0.372	0.201	0.0105	0.105	0.00300	0.323	0.260	0.0035	0.035	0.000999
6.	31.0	0.450	0.292	0.010	0.10	0.00322	0.382	0.342	0.003	0.03	0.000969
7.	29.0	0.362	0.194	0.0095	0.095	0.00327	0.342	0.292	0.0035	0.035	0.000862
8.	30.0	0.492	0.342	0.009	0.09	0.00300	0.323	0.222	0.003	0.03	0.000999
Mean	32.43					0.00316					0.000979
S.D.	±2.845					±0.000180					±0.0000707

Note: Aliquot taken = 0-1 ml.
Water added = 0.9 ml.

APPENDIX TABLE 7. 4th Instar. One-day-old.

No. of sample.	wt. of body (mg)	Acid phosphatase activity					Alkaline phosphatase activity				
		Optical density of		Pi in the		Pi per mg. wt. of body (mg)	Optical density of		Pi in the		Pi per mg. wt. of body (mg)
		Exp.	Control	aliquot (mg)	whole body (mg)		Exp.	Control	aliquot (mg)	whole body (mg)	
1.	37.0	0.420	0.201	0.0135	0.135	0.00364	0.272	0.191	0.0045	0.045	0.00121
2.	36.0	0.414	0.211	0.012	0.12	0.00333	0.280	0.211	0.004	0.04	0.00111
3.	37.5	0.432	0.229	0.012	0.12	0.00320	0.292	0.194	0.0045	0.045	0.00120
4.	35.0	0.450	0.240	0.0125	0.125	0.00357	0.297	0.233	0.004	0.04	0.00114
5.	38.0	0.432	0.201	0.014	0.14	0.00368	0.323	0.248	0.0045	0.045	0.00118
6.	39.0	0.432	0.211	0.013	0.13	0.00333	0.342	0.264	0.0045	0.045	0.00115
7.	38.0	0.414	0.215	0.012	0.12	0.00375	0.444	0.414	0.004	0.04	0.00126
8.	39.0	0.432	0.211	0.0135	0.135	0.00346	0.292	0.211	0.0045	0.045	0.00115
Mean	36.88					0.00349					0.00117
S.D. ±	2.344					± 0.000196					± 0.0000447

Note: Aliquot taken = 0.1 ml.
Water added = 0.9 ml.

APPENDIX TABLE 8. 4th Instar. Two-day-old

No. of sample.	wt. of body (mg)	Acid phosphatase activity					Alkaline phosphatase activity				
		Optical density of		Pi in the		Pi per mg. wt. of body (mg)	Optical density of		Pi in the		Pi per mg. wt. of body (mg)
		Exp.	Control	aliquot (mg)	whole body (mg)		Exp.	Control	aliquot (mg)	whole body (mg)	
1.	39.0	0.577	0.240	0.02	0.20	0.00512	0.414	0.211	0.012	0.12	0.00307
2.	38.0	0.509	0.191	0.0195	0.195	0.00513	0.403	0.215	0.0115	0.115	0.00302
3.	41.0	0.530	0.211	0.0195	0.195	0.00475	0.409	0.233	0.011	0.11	0.00268
4.	43.0	0.569	0.211	0.022	0.22	0.00511	0.420	0.194	0.0135	0.135	0.00312
5.	42.0	0.569	0.233	0.021	0.21	0.00488	0.460	0.260	0.0125	0.125	0.00290
6.	40.0	0.585	0.201	0.0205	0.205	0.00525	0.495	0.292	0.012	0.12	0.00307
7.	45.0	0.602	0.201	0.0215	0.215	0.00477	0.462	0.252	0.013	0.13	0.00288
8.	44.5	0.620	0.204	0.0225	0.225	0.00505	0.450	0.233	0.0135	0.135	0.00303
Mean	41.56					0.00501					0.00297
S.D.	± 2.691					± 0.000184					± 0.000146

Note: Aliquot taken = 0.1 ml.
Water added = 0.9 ml.

APPENDIX TABLE 9. 4th Instar. Three-day-old.

No. of sample	wt. of body (mg)	Acid phosphatase activity					Alkaline phosphatase activity				
		Optical density of		Pi in the		Pi per mg. wt. of body (mg)	Optical density of		Pi in the		Pi per mg. wt. of body (mg)
		Exp.	Control	aliquot (mg)	whole body (mg)		Exp.	Control	aliquot (mg)	whole body (mg)	
1.	45.0	0.611	0.194	0.025	0.25	0.00561	0.462	0.201	0.016	0.16	0.00355
2.	49.0	0.721	0.260	0.03	0.30	0.00612	0.495	0.211	0.017	0.17	0.00346
3.	46.0	0.648	0.211	0.027	0.27	0.00586	0.475	0.222	0.0155	0.155	0.00336
4.	44.0	0.638	0.240	0.025	0.25	0.00568	0.495	0.244	0.0155	0.155	0.00352
5.	48.5	0.648	0.201	0.275	0.275	0.00572	0.502	0.233	0.0165	0.165	0.00342
6.	49.0	0.688	0.194	0.0305	0.305	0.00622	0.509	0.211	0.0185	0.185	0.00377
7.	42.0	0.680	0.222	0.0245	0.245	0.00583	0.432	0.194	0.0145	0.145	0.00345
8.	41.0	0.620	0.201	0.0255	0.255	0.00621	0.495	0.244	0.015	0.15	0.00365
Mean	45.60					0.00591					0.00352
S.D.	± 3.07					± 0.000245					± 0.000121

Note: Aliquot taken = 0.1 ml.
Water added = 0.9 ml.

APPENDIX TABLE 10. 4th Instar. Four-day-old.

No. of sample.	Wt. of body (mg)	Acid phosphatase activity					Alkaline phosphatase activity				
		Optical density of		Pi in the		Pi per mg. wt. of body (mg)	Optical density of		Pi in the		Pi per mg. wt. of body (mg)
		Expt.	Control	aliquot (mg)	whole body (mg)		Exp.	Control	aliquot (mg)	whole body (mg)	
1.	45.0	0.516	0.191	0.0195	0.195	0.00433	0.495	0.342	0.009	0.09	0.00019
2.	47.0	0.589	0.211	0.0215	0.215	0.00457	0.414	0.240	0.01	0.10	0.00212
3.	49.0	0.620	0.215	0.025	0.25	0.00510	0.432	0.260	0.0105	0.105	0.00214
4.	51.0	0.630	0.222	0.025	0.25	0.00485	0.450	0.280	0.0105	0.105	0.00203
5.	55.0	0.638	0.233	0.025	0.25	0.00454	0.403	0.211	0.0115	0.115	0.00209
6.	54.0	0.648	0.280	0.023	0.23	0.00425	0.372	0.191	0.0105	0.105	0.00194
7.	56.0	0.611	0.211	0.024	0.24	0.00428	0.392	0.264	0.011	0.11	0.00196
8.	48.0	0.594	0.222	0.0225	0.225	0.00468	0.509	0.342	0.01	0.10	0.00208
Mean	50.62					0.00458					0.00204
S.D.	±4.049					±0.000295					±0.0000762

Note: Aliquot taken 0.1 ml.
Water added 0.9 ml.

APPENDIX TABLE 11. 5th Instar. Zero-day-old.

No. of sample	Wt. of body (mg)	Acid phosphatase activity					Alkaline phosphatase activity				
		Optical density of		Pi in the		Pi per mg. wt. of body (mg)	Optical density of		Pi in the		Pi per mg. wt. of body (mg)
		Exp.	Control	aliquot (mg)	whole body (mg)		Exp.	Control	aliquot (mg)	whole body (mg)	
1.	49.0	0.495	0.194	0.019	0.19	0.00387	0.332	0.240	0.005	0.05	0.00102
2.	51.0	0.561	0.211	0.021	0.21	0.00411	0.342	0.264	0.005	0.05	0.00098
3.	54.0	0.585	0.233	0.0215	0.215	0.00398	0.392	0.301	0.0055	0.055	0.00101
4.	56.5	0.594	0.222	0.0225	0.225	0.00401	0.414	0.314	0.006	0.06	0.00107
5.	48.0	0.509	0.191	0.0195	0.195	0.00406	0.432	0.342	0.005	0.05	0.00104
6.	52.0	0.602	0.264	0.0205	0.205	0.00394	0.495	0.414	0.005	0.05	0.000961
7.	53.0	0.648	0.292	0.022	0.22	0.00411	0.337	0.244	0.0055	0.055	0.00102
8.	55.0	0.638	0.222	0.0225	0.225	0.00383	0.332	0.233	0.006	0.06	0.00103
Mean	52.06					0.004					0.00101
S.D.	± 3.39					± 0.0000934					± 0.0000345

Note: Aliquot taken = 0.1 ml.
Water added = 0.9 ml.

APPENDIX TABLE 12. 5th Instar. One-day-old.

No. of sample	Wt. of body (mg)	Acid phosphatase activity					Alkaline phosphatase activity				
		Optical density of		Pi in the		Pi per mg. wt. of body (mg)	Optical density of		Pi in the		Pi per mg. wt. of body (mg)
		Exp.	Control	aliquot (mg)	whole body (mg)		Exp.	Control	aliquot (mg)	whole body (mg)	
1.	52.0	0.694	0.211	0.023	0.23	0.00442	0.372	0.248	0.0075	0.075	0.00144
2.	54.0	0.689	0.233	0.0245	0.245	0.00453	0.342	0.211	0.008	0.08	0.00148
3.	56.0	0.648	0.252	0.025	0.25	0.00446	0.475	0.332	0.0085	0.085	0.00151
4.	59.0	0.638	0.194	0.027	0.27	0.00457	0.444	0.292	0.009	0.09	0.00152
5.	53.5	0.620	0.233	0.024	0.24	0.00448	0.535	0.414	0.008	0.08	0.00149
6.	54.0	0.648	0.244	0.0245	0.245	0.00453	0.495	0.362	0.008	0.08	0.00148
7.	59.5	0.638	0.211	0.026	0.26	0.00436	0.482	0.342	0.0085	0.085	0.00142
8.	58.0	0.620	0.191	0.0265	0.265	0.00456	0.432	0.292	0.009	0.09	0.00155
Mean	56.75 ± 2.803					0.00449 ± 0.0000731					0.00149 ± 0.0000415

Notes: Aliquot taken = 0.1 ml.
Water added = 0.9 ml.

APPENDIX TABLE 13. 5th Instar Two-day-old.

No. of sample.	Wt. of body (mg)	Acid phosphatase activity					Alkaline phosphatase activity				
		Optical density of		Pi in the		Pi per mg. wt. of body (mg)	Optical density of		Pi in the		Pi per mg. wt. of body (mg)
		Exp.	Control	aliquot (mg)	whole body (mg)		Exp.	Control	aliquot (mg)	whole body (mg)	
1.	58.0	0.733	0.272	0.0285	0.285	0.00491	0.495	0.211	0.017	0.17	0.00293
2.	59.0	0.824	0.332	0.03	0.30	0.00509	0.495	0.194	0.018	0.18	0.00305
3.	61.0	0.838	0.362	0.03	0.30	0.00491	0.545	0.240	0.018	0.18	0.00295
4.	56.0	0.611	0.197	0.028	0.28	0.00499	0.516	0.240	0.0165	0.165	0.00294
5.	62.0	0.745	0.252	0.0305	0.305	0.00491	0.576	0.280	0.0185	0.185	0.00298
6.	60.0	0.745	0.260	0.03	0.3	0.00499	0.638	0.340	0.018	0.18	0.00300
7.	66.0	0.824	0.244	0.035	0.35	0.00530	0.561	0.222	0.0205	0.205	0.00310
8.	65.5	0.721	0.201	0.0315	0.315	0.00480	0.509	0.204	0.019	0.19	0.00290
Mean	60.93					0.00499					0.00298
S.D.	± 3.489					± 0.000149					± 0.0000874

Note: Aliquot taken = 0.1 ml.
Water added = 0.9 ml.

APPENDIX TABLE 14. 5th Instar. Three-day-old.

No. of sample.	Wt. of body (mg)	Acid phosphatase activity					Alkaline phosphatase activity				
		Optical density of		Pi in the		Pi per mg. wt. of body (mg)	Optical density of		Pi in the		Pi per mg. wt. of body (mg)
		Exp.	Control	aliquot (mg)	whole body (mg)		Exp.	Control	aliquot (mg)	whole body (mg)	
1.	61.0	0.688	0.240	0.0275	0.275	0.00450	0.403	0.197	0.0125	0.125	0.00204
2.	66.5	0.870	0.409	0.03	0.30	0.00451	0.432	0.204	0.0135	0.135	0.00203
3.	65.0	0.688	0.194	0.0285	0.285	0.00438	0.444	0.233	0.013	0.13	0.00200
4.	60.0	0.648	0.201	0.0275	0.275	0.00458	0.414	0.204	0.0125	0.125	0.00208
5.	59.0	0.620	0.121	0.026	0.26	0.00440	0.475	0.292	0.0115	0.115	0.00194
6.	62.0	0.678	0.233	0.028	0.28	0.00444	0.392	0.191	0.0125	0.125	0.00198
7.	68.0	0.721	0.211	0.031	0.31	0.00455	0.462	0.233	0.014	0.14	0.00205
8.	61.5	0.638	0.197	0.027	0.27	0.00439	0.414	0.201	0.0125	0.125	0.00203
Mean	62.98					0.00447					0.00202
S.D.	±2.225					±0.0000764					±0.0000486

Note: Aliquot taken = 0.1 ml.
Water added = 0.9 ml.

APPENDIX TABLE 15. 5th Instar Four-day-old

No. of sample	wt. of body (mg)	Acid phosphatase activity					Alkaline phosphatase activity				
		Optical density of		Pi in the		Pi per mg. wt. of body (mg)	Optical density of		Pi in the		Pi per mg. wt. of body (mg)
		Exp.	Control	aliquot (mg)	whole body (mg)		Exp.	Control	aliquot (mg)	whole body (mg)	
1.	68.0	0.502	0.201	0.0165	0.155	0.00227	0.382	0.248	0.008	0.08	0.00117
2.	72.0	0.509	0.222	0.0175	0.175	0.00243	0.495	0.342	0.009	0.09	0.00124
3.	70.5	0.530	0.201	0.017	0.17	0.00241	0.523	0.382	0.0085	0.085	0.00120
4.	73.0	0.569	0.292	0.0175	0.175	0.00239	0.392	0.240	0.009	0.09	0.00123
5.	69.0	0.509	0.240	0.0165	0.165	0.00239	0.332	0.194	0.008	0.08	0.00115
6.	74.0	0.495	0.197	0.0180	0.18	0.00243	0.392	0.237	0.009	0.09	0.00121
7.	61.0	0.462	0.226	0.0145	0.145	0.00237	0.342	0.222	0.0075	0.075	0.00123
8.	66.0	0.523	0.248	0.0165	0.165	0.00249	0.362	0.233	0.008	0.08	0.00121
Mean	69.18					0.00240					0.00121
S.D.	±4.242					±0.0000623					±0.0000288

Note: Aliquot taken=0.1 ml.
water added =0.9 ml.

APPENDIX TABLE 16. Zero-day-old female

No. of samples	Wt. of insect (mg)	Acid phosphatase activity					Alkaline phosphatase activity				
		Optical density of		Pi in the		Pi per mg. wt. of body	Optical density of		Pi in the		Pi per mg. wt. of body
		Exp.	Control	aliquot (mg)	whole body (mg)	(mg)	Exp.	Control	aliquot (mg)	whole body (mg)	(mg)
1.	68.0	0.516	0.342	0.010	0.10	0.00147	0.305	0.215	0.0045	0.045	0.000660
2.	67.5	0.352	0.181	0.0105	0.105	0.00155	0.264	0.181	0.005	0.05	0.000730
3.	68.5	0.414	0.233	0.0105	0.105	0.00153	0.280	0.197	0.005	0.05	0.000730
4.	73.00	0.414	0.181	0.011	0.11	0.00150	0.432	0.342	0.005	0.05	0.000680
5.	62.5	0.342	0.181	0.0095	0.095	0.00152	0.292	0.211	0.0055	0.055	0.000880
6.	65.5	0.347	0.204	0.0095	0.095	0.00145	0.382	0.297	0.0045	0.045	0.000680
7.	75.0	0.432	0.184	0.011	0.11	0.00146	0.372	0.280	0.005	0.05	0.000660
8.	68.5	0.420	0.181	0.0105	0.105	0.00153	0.305	0.211	0.0055	0.055	0.000800
9.	72.5	0.495	0.292	0.012	0.12	0.00165	0.444	0.362	0.005	0.05	0.000680
Mean		68.89				0.00151					0.000722
S.D.		± 3.940				± 0.0000605					± 0.0000743

Note: Aliquot taken = 0.1 ml.
Water added = 0.9 ml.

APPENDIX TABLE 17. One-day-old female

No. of samples	Wt. of insect (mg)	Acid phosphatase activity					Alkaline phosphatase activity				
		Optical density of		Pi in the		Pi per mg. wt. of body (mg)	Optical density of		Pi in the		Pi per mg. wt. of body (mg)
		Exp.	Control	aliquot (mg)	whole body (mg)		Exp.	Control	aliquot (mg)	whole body (mg)	
1.	78.0	0.444	0.201	0.015	0.15	0.00192	0.292	0.184	0.007	0.07	0.00089
2.	73.5	0.432	0.184	0.0155	0.155	0.00210	0.332	0.211	0.007	0.07	0.00095
3.	69.0	0.432	0.211	0.013	0.13	0.00188	0.314	0.233	0.005	0.05	0.00072
4.	70.5	0.530	0.284	0.015	0.15	0.00212	0.323	0.215	0.0065	0.065	0.00096
5.	68.0	0.420	0.215	0.0125	0.125	0.00183	0.352	0.264	0.0055	0.055	0000080
6.	79.0	0.475	0.208	0.017	0.17	0.00215	0.332	0.203	0.0075	0.075	0.00069
7.	67.0	0.432	0.233	0.0125	0.125	0.00186	0.392	0.292	0.0055	0.055	0.00082
8.	68.0	0.495	0.264	0.014	0.14	0.00205	0.450	0.342	0.0065	0.065	0.00095
9.	67.0	0.469	0.240	0.0135	0.135	0.00201	0.611	0.509	0.006	0.06	0.00089
Mean		71.11				0.00199					0.000846
S.D.		± 4.655				±0.000121					±0.0000952

Note: Aliquot taken = 0.1 ml.
Water added = 0.9 ml.

APPENDIX TABLE 18. Two-day-old female

No. of samples	Wt. of insect (mg)	Acid phosphatase activity					Alkaline phosphatase activity				
		Optical density of		Pi in the		Pi per mg. wt. of body (mg)	Optical density of		Pi in the		Pi per mg. wt. of body (mg)
		Expt.	Control	aliquot (mg)	Whole body (mg)		Exp.	Control	aliquot (mg)	Whole body (mg)	
1.	83.0	0.482	0.191	0.0175	0.175	0.00210	0.382	0.181	0.0125	0.125	0.0015
2.	74.0	0.469	0.211	0.0155	0.155	0.00209	0.382	0.194	0.0110	0.110	0.00148
3.	78.0	0.538	0.280	0.0150	0.150	0.00192	0.438	0.240	0.0115	0.115	0.00147
4.	73.0	0.444	0.181	0.0160	0.160	0.00219	0.382	0.191	0.0115	0.115	0.00157
5.	76.0	0.482	0.244	0.0150	0.150	0.00192	0.420	0.244	0.0105	0.105	0.00138
6.	84.0	0.509	0.204	0.0185	0.185	0.00220	0.502	0.280	0.013	0.13	0.00154
7.	72.5	0.469	0.233	0.0145	0.45	0.00200	0.502	0.332	0.0105	0.105	0.00145
8.	72.0	0.530	0.292	0.0150	0.150	0.00208	0.482	0.280	0.012	0.12	0.00166
9.	74.5	0.430	0.204	0.0180	0.150	0.00201	0.403	0.218	0.011	0.11	0.00147
Mean		76.33				0.00205					0.00150
S.D.		± 4.465				± 0.0001028					± 0.0000799

Note: Aliquot taken = 0.1 ml.
Water added = 0.9 ml.

APPENDIX TABLE 19. Three-day-old female

No. of samples	wt. of insect (mg)	Acid phosphatase activity					Alkaline phosphatase activity				
		Optical density of		Pi in the		Pi per mg. wt. of body (mg)	Optical density of		Pi in the		Pi per mg. wt. of body (mg)
		Exp.	Control	aliquot (mg)	Whole body (mg)		Exp.	Control	aliquot (mg)	Whole body (mg)	
1.	92.0	0.638	0.184	0.0285	0.285	0.00309	0.450	0.181	0.0165	0.165	0.00179
2.	82.0	0.638	0.194	0.0270	0.270	0.00329	0.495	0.252	0.0150	0.150	0.00182
3.	79.0	0.620	0.211	0.0245	0.245	0.00310	0.403	0.184	0.0135	0.135	0.00170
4.	89.0	0.721	0.252	0.0290	0.290	0.00325	0.482	0.211	0.0165	0.165	0.00179
5.	68.0	0.620	0.280	0.0205	0.205	0.00301	0.382	0.204	0.0115	0.115	0.00169
6.	77.0	0.629	0.211	0.0255	0.255	0.00330	0.450	0.211	0.0145	0.145	0.00188
7.	79.00	0.595	0.215	0.0240	0.240	0.00303	0.444	0.222	0.0135	0.135	0.00170
8.	75.5	0.602	0.184	0.0255	0.255	0.00337	0.495	0.280	0.0140	0.140	0.00186
9.	79.0	0.620	0.215	0.0250	0.250	0.00316	0.523	0.292	0.0140	0.140	0.00177
Mean		80.05				0.00314					0.00177
S.D.		± 7.117				± 0.000134					±0.0000699

Note: Aliquot taken = 0.1 ml.
Water added = 0.9 ml.

APPENDIX TABLE 20. Four-day-old female

No. of samples	Wt. of insect (mg)	Acid phosphatase activity					Alkaline phosphatase activity				
		Optical density of		Pi in the		Pi per mg. wt. of body (mg)	Optical density of		Pi in the		Pi per mg. wt. of body (mg)
		Exp.	Control	aliquot (mg)	Whole body (mg)		Exp.	Control	aliquot (mg)	Whole body (mg)	
1.	87.0	0.745	0.204	0.0340	0.340	0.00390	0.482	0.184	0.0185	0.185	0.00212
2.	82.0	0.745	0.181	0.0350	0.350	0.00426	0.516	0.204	0.0185	0.185	0.00225
3.	91.0	0.782	0.211	0.0355	0.355	0.00390	0.523	0.194	0.0195	0.195	0.00214
4.	96.0	0.854	0.201	0.040	0.40	0.00416	0.577	0.211	0.0215	0.215	0.00230
5.	78.0	0.745	0.260	0.030	0.30	0.00384	0.516	0.240	0.0165	0.165	0.00211
6.	84.0	0.648	0.181	0.0345	0.345	0.00410	0.495	0.184	0.0190	0.190	0.00226
7.	81.5	0.678	0.194	0.030	0.30	0.00368	0.530	0.252	0.0175	0.175	0.00214
8.	84.5	0.782	0.211	0.035	0.35	0.00414	0.585	0.272	0.0190	0.190	0.00224
9.	88.5	0.870	0.292	0.035	0.35	0.00395	0.523	0.201	0.0195	0.195	0.00220
Mean		85.83				0.00399					0.00219
S.D.		± 5.460				± 0.000184					±0.0000700

Note: Aliquot taken = 0.1 ml.
Water added = 0.9 ml.

APPENDIX TABLE 21. Five-day-old female

No. of samples	Wt. of insect (mg)	Acid phosphatase activity					Alkaline phosphatase activity				
		Optical density of		Pi in the		Pi per mg. wt. of body (mg)	Optical density of		Pi in the		Pi per mg. wt. of body (mg)
		Exp.	Control	aliquot (mg)	Whole body (mg)		Exp.	Control	aliquot (mg)	Whole body (mg)	
1.	102.0	0.903	0.201	0.0435	0.435	0.00426	0.629	0.215	0.0255	0.255	0.00250
2.	98.0	0.939	0.264	0.0420	0.420	0.00428	0.611	0.191	0.0250	0.250	0.00255
3.	79.0	0.782	0.184	0.0370	0.370	0.00468	0.648	0.292	0.0220	0.220	0.00278
4.	96.0	0.870	0.211	0.0410	0.410	0.00427	0.629	0.232	0.0245	0.245	0.00255
5.	88.0	0.838	0.201	0.0395	0.395	0.00448	0.569	0.184	0.0235	0.235	0.00267
6.	89.0	0.824	0.194	0.0380	0.380	0.00426	0.561	0.194	0.0225	0.225	0.00252
7.	85.0	0.824	0.204	0.0385	0.385	0.00452	0.569	0.201	0.0225	0.225	0.00264
8.	90.0	0.838	0.211	0.0390	0.390	0.00433	0.620	0.240	0.0230	0.230	0.00255
9.	92.0	0.886	0.330	0.0400	0.400	0.00444	0.611	0.232	0.0235	0.235	0.00255
Mean		91.05				0.00439					0.00258
S.D.		± 6.930				± 0.000148					± 0.0000897

Note: Aliquot taken = 0.1 ml.
Water added = 0.9 ml.

APPENDIX TABLE 22. Six-day-old female

No. of samples	Wt. of insect (mg)	Acid phosphatase activity					Alkaline phosphatase activity				
		Optical density of		Pi in the		Pi per mg. wt. of body (mg)	Optical density of		Pi in the		Pi per mg. wt. of body (mg)
		Exp.	Control	aliquot (mg)	Whole body (mg)		Exp.	Control	aliquot (mg)	Whole body (mg)	
1.	137.0	1.347	0.194	0.0725	0.725	0.00529	0.745	0.197	0.0330	0.330	0.00240
2.	121.0	1.301	0.201	0.0655	0.655	0.00541	0.699	0.211	0.0295	0.295	0.00243
3.	97.0	1.260	0.204	0.0560	0.560	0.00577	0.629	0.215	0.0255	0.255	0.00262
4.	102.0	1.046	0.252	0.0550	0.550	0.00539	0.611	0.244	0.0250	0.250	0.00245
5.	104.0	1.187	0.280	0.0550	0.550	0.00528	0.602	0.181	0.0255	0.255	0.00245
6.	98.0	1.126	0.240	0.0550	0.550	0.00561	0.620	0.211	0.0250	0.250	0.00255
7.	92.0	1.076	0.222	0.0525	0.525	0.00570	0.595	0.194	0.0240	0.240	0.00260
8.	123.0	1.222	0.194	0.0640	0.640	0.00520	0.602	0.184	0.0290	0.290	0.00235
9.	98.0	1.126	0.233	0.0560	0.560	0.005714	0.629	0.215	0.0255	0.255	0.00260
Mean		108.00				0.00548					0.00249
S.D.		±15.264				±0.000215					±0.000093

Note: Aliquot taken = 0.1 ml.
Water added = 0.9 ml.

APPENDIX TABLE 23. Seven-day-old female

No. of samples	Wt. of insect (mg)	Acid phosphatase activity					Alkaline phosphatase activity				
		Optical density of		Pi in the		Pi per mg. wt. of body (mg)	Optical density of		Pi in the		Pi per mg. wt. of body (mg)
		Exp.	Control	aliquot (mg)	Whole body (mg)		Exp.	Control	aliquot (mg)	Whole body (mg)	
1.	87.0	0.787	0.197	0.0360	0.360	0.00413	0.620	0.342	0.0175	0.175	0.00201
2.	89.0	0.782	0.215	0.0350	0.350	0.00393	0.469	0.194	0.0170	0.170	0.00191
3.	78.0	0.810	0.280	0.330	0.330	0.00423	0.509	0.208	0.0180	0.180	0.00230
4.	101.0	0.886	0.240	0.0390	0.390	0.00386	0.569	0.233	0.0160	0.160	0.00158
5.	98.0	0.824	0.211	0.0370	0.370	0.00377	0.594	0.284	0.0190	0.190	0.00193
6.	78.0	0.979	0.462	0.0310	0.310	0.00407	0.509	0.252	0.0155	0.155	0.00203
7.	75.0	0.658	0.184	0.0290	0.290	0.00386	0.475	0.233	0.0145	0.145	0.00193
8.	75.5	0.648	0.181	0.0285	0.285	0.00377	0.482	0.233	0.0155	0.155	0.00205
9.	73.0	0.648	0.201	0.0280	0.280	0.00383	0.450	0.211	0.0145	0.145	0.00198
Mean		84.61				0.00382					0.00197
S.D.		± 12.63				± 0.000397					± 0.000186

Note: Aliquot taken = 0.1 ml.
Water added = 0.9 ml.

APPENDIX TABLE 24. Eight-day-old female

No. of samples	Wt. of insect (mg)	Acid phosphatase activity					Alakline phosphatase activity				
		Optical density of		Pi in the		Pi per mg. wt. of body (mg)	Optical density of		Pi in the		Pi per mg. wt. of body (mg)
		Exp.	Control	aliquot (mg)	Whole body (mg)		Exp.	Control	aliquot (mg)	Whole body (mg)	
1.	89.0	0.629	0.211	0.0255	0.255	0.00286	0.420	0.194	0.0135	0.135	0.00151
2.	98.0	0.629	0.194	0.0265	0.265	0.00270	0.450	0.211	0.0145	0.145	0.00147
3.	82.0	0.602	0.233	0.0225	0.225	0.00274	0.432	0.233	0.0120	0.120	0.00146
4.	86.0	0.620	0.215	0.0245	0.245	0.00284	0.502	0.284	0.0135	0.135	0.00157
5.	78.0	0.553	0.181	0.0225	0.225	0.00288	0.488	0.293	0.0120	0.120	0.00153
6.	97.0	0.620	0.201	0.0250	0.250	0.00257	0.495	0.264	0.0140	0.140	0.00144
7.	96.0	0.638	0.222	0.0255	0.255	0.00265	0.509	0.272	0.0145	0.145	0.00151
8.	76.0	0.511	0.260	0.0215	0.215	0.00282	0.516	0.319	0.0115	0.115	0.00151
9.	72.0	0.629	0.292	0.0205	0.205	0.00284	0.585	0.342	0.0105	0.105	0.00145
Mean		86.00				0.00276					0.00149
S.D.		± 9.682				±					± 0.0000424

Note: Aliquot taken = 0.1 ml.
Water added = 0.9 ml.

APPENDIX TABLE 25. Nine-day-old female

No. of sample.	Wt. of insect (mg)	Acid phosphatase activity					Alkaline phosphatase activity				
		Optical density of		Pi in the		Pi per mg. wt. of body (mg)	Optical density of		Pi in the		Pi per mg. wt. of body (mg)
		Exp.	Control	aliquot (mg)	Whole body (mg)		Exp.	Control	aliquot (mg)	Whole body (mg)	
1.	102.0	0.782	0.211	0.0350	0.350	0.00343	0.420	0.197	0.0130	0.130	0.00127
2.	96.0	0.824	0.260	0.0340	0.340	0.00354	0.426	0.191	0.0125	0.125	0.00130
3.	89.0	0.684	0.194	0.0305	0.305	0.00342	0.392	0.211	0.0115	0.115	0.00129
4.	86.0	0.824	0.323	0.0300	0.300	0.00348	0.414	0.272	0.0115	0.115	0.00133
5.	78.0	0.638	0.184	0.0285	0.285	0.00365	0.444	0.272	0.0105	0.105	0.00134
6.	86.0	0.668	0.181	0.0290	0.290	0.00337	0.462	0.292	0.0105	0.105	0.00122
7.	91.0	0.782	0.240	0.0330	0.330	0.00362	0.420	0.211	0.0125	0.125	0.00137
8.	119.0	0.921	0.284	0.0395	0.395	0.00332	0.444	0.208	0.0145	0.145	0.00121
9.	96.0	0.782	0.226	0.0345	0.345	0.00359	0.509	0.292	0.0135	0.135	0.00140
Mean		93.66				0.00349					0.00128
S.D.		± 11.80			±	0.000115				±	0.0000609

Note: Aliquot taken = 0.1 ml.
Water added = 0.9 ml.

APPENDIX TABLE 26. Ten-day-old female

No. of sample.	Wt. of insect (mg)	Acid phosphatase activity					Alkaline phosphatase activity				
		Optical density of		Pi in the		Pi per mg. wt. of body (mg)	Optical density of		Pi in the		Pi per mg. wt. of body (mg)
		Exp.	Control	aliquot (mg)	whole body (mg)		Exp.	Control	aliquot (mg)	Whole body (mg)	
1.	137.0	1.126	0.211	0.0550	0.550	0.00401	0.585	0.181	0.0245	0.245	0.00178
2.	102.0	0.886	0.201	0.0415	0.415	0.00406	0.509	0.211	0.0185	0.185	0.00181
3.	98.0	0.870	0.202	0.0385	0.385	0.00392	0.545	0.248	0.0175	0.175	0.00178
4.	89.0	0.782	0.194	0.0365	0.365	0.00410	0.462	0.204	0.0160	0.160	0.00179
5.	88.0	0.824	0.233	0.0360	0.360	0.00409	0.482	0.215	0.0160	0.160	0.00181
6.	99.5	0.921	0.292	0.0385	0.385	0.00386	0.523	0.240	0.0175	0.175	0.00175
7.	98.5	1.301	0.648	0.0400	0.400	0.00406	0.482	0.181	0.0180	0.180	0.00182
8.	104.0	1.022	0.352	0.0405	0.405	0.00389	0.611	0.301	0.0185	0.185	0.00177
9.	85.0	0.921	0.372	0.0335	0.335	0.00394	0.444	0.194	0.0150	0.150	0.00176
Mean		100.11				0.00399					0.00178
S.D.		± 15.35				± 0.0000912					±0.0000240

Note: Aliquot taken = 0.1 ml.
water added = 0.9 ml.

APPENDIX TABLE 27. Eleven-day-old female

No. of female.	Wt. of insect (mg)	Acid phosphatase activity					Alkaline phosphatase activity				
		Optical density of		Pi in the		Pi per mg. wt. of body (mg)	Optical density of		Pi in the		Pi per mg. wt. of body (mg)
		Exp.	control	aliquot (mg)	whole body (mg)		Exp.	control	aliquot (mg)	whole (mg)	
1.	126.0	1.187	0.201	0.0595	0.595	0.00472	0.648	0.201	0.0270	0.270	0.00214
2.	127.0	1.301	0.332	0.0600	0.600	0.00472	0.638	0.194	0.0275	0.275	0.00216
3.	93.0	0.979	0.226	0.0465	0.465	0.00500	0.561	0.211	0.0215	0.215	0.00231
4.	89.0	0.921	0.240	0.0415	0.415	0.00466	0.545	0.233	0.0190	0.190	0.00213
5.	83.5	1.272	0.382	0.0405	0.405	0.00485	0.516	0.201	0.0185	0.185	0.00221
6.	96.5	1.022	0.240	0.0470	0.470	0.00487	0.620	0.260	0.0215	0.215	0.00222
7.	105.0	0.979	0.184	0.0490	0.490	0.00466	0.629	0.264	0.0225	0.225	0.00214
8.	102.0	1.022	0.233	0.0480	0.480	0.00470	0.638	0.280	0.0220	0.220	0.00215
9.	102.0	1.022	0.201	0.0495	0.495	0.00485	0.594	0.222	0.0225	0.225	0.00220
Mean		102.66				0.00478					0.00218
S.D.		± 15.118				± 0.000116					±0.0000576

Note: Aliquot taken = 0.1 ml.
Water added = 0.9 ml.

APPENDIX TABLE 28. Zero-day-old male

No. of sample.	Wt. of insect (mg)	Acid phosphatase activity					Alkaline phosphatase activity				
		Optical density of		Pi in the		Pi per mg. wt. of body (mg)	Optical density of		Pi in the		Pi per mg. wt. of body (mg)
		Exp.	control	aliquot (mg)	whole body (mg)		Exp.	control	aliquot (mg)	whole body (mg)	
1.	38.0	0.382	0.264	0.008	0.08	0.00210	0.233	0.194	0.0025	0.025	0.000650
2.	47.0	0.509	0.342	0.0105	0.105	0.00223	0.264	0.211	0.003	0.03	0.000630
3.	36.0	0.314	0.194	0.0075	0.075	0.00208	0.280	0.252	0.0025	0.025	0.000694
4.	42.0	0.426	0.276	0.0095	0.095	0.00226	0.362	0.310	0.003	0.03	0.000714
5.	45.0	0.488	0.342	0.0095	0.095	0.00211	0.229	0.184	0.003	0.03	0.000666
6.	32.0	0.314	0.211	0.0065	0.065	0.00243	0.310	0.284	0.002	0.02	0.000625
7.	39.0	0.332	0.181	0.009	0.09	0.00230	0.342	0.292	0.003	0.03	0.000769
8.	33.0	0.502	0.362	0.008	0.08	0.00242	0.362	0.323	0.002	0.02	0.000606
9.	46.0	0.352	0.211	0.009	0.09	0.00195	0.215	0.152	0.0035	0.035	0.000760
Mean		39.777				0.00216					0.000679
S.D.		± 5.562				± 0.000148					±0.000059

Note: Aliquot taken = 0.1 ml.
Water added = 0.9 ml.

APPENDIX TABLE 29. One-day-old male

No. of sample	Wt. of insect (mg)	Acid phosphatase activity					Alkaline phosphatase activity				
		Optical density of		Pi in the		Pi per mg. wt. of body (mg)	Optical density of		Pi in the		Pi per mg. wt. of body (mg)
		Exp.	control	aliquot (mg)	whole body (mg)		Exp.	control	aliquot (mg)	whole body (mg)	
1.	31.0	0.462	0.342	0.0075	0.075	0.00241	0.283	0.244	0.0035	0.035	0.00112
2.	33.0	0.292	0.181	0.007	0.07	0.00212	0.362	0.292	0.004	0.04	0.00121
3.	40.0	0.488	0.332	0.010	0.10	0.00250	0.362	0.280	0.005	0.05	0.00125
4.	42.0	0.420	0.264	0.0095	0.095	0.00226	0.194	0.114	0.005	0.05	0.00119
5.	35.0	0.323	0.201	0.0085	0.085	0.00242	0.409	0.342	0.004	0.04	0.00114
6.	29.0	0.462	0.342	0.0072	0.072	0.00241	0.426	0.342	0.004	0.04	0.00137
7.	34.5	0.538	0.409	0.008	0.08	0.00231	0.432	0.372	0.0035	0.035	0.00101
8.	37.0	0.516	0.372	0.0085	0.085	0.00229	0.495	0.420	0.004	0.04	0.00108
9.	36.0	0.495	0.342	0.009	0.09	0.00250	0.488	0.409	0.005	0.05	0.00138
Mean		35.277				0.00235					0.00119
S.D.		± 4.101				± 0.000123					± 0.0001246

Note: Aliquot taken = 0.1 ml.
Water added = 0.9 ml.

APPENDIX TABLE 30. Two-day-old male

No. of sample	Wt. of insect (mg)	Acid phosphatase activity					Alkaline phosphatase activity				
		Optical density of		Pi in the		Pi per mg. wt. of body (mg)	Optical density of		Pi in the		Pi per mg. wt. of body (mg)
		Exp.	control	aliquot (mg)	whole body (mg)		Exp.	control	aliquot (mg)	whole body (mg)	
1.	49.5	0.620	0.204	0.026	0.26	0.00525	0.342	0.204	0.009	0.09	0.00181
2.	51.0	0.594	0.184	0.025	0.25	0.00490	0.332	0.184	0.009	0.09	0.00176
3.	39.0	0.561	0.222	0.0205	0.205	0.00525	0.392	0.284	0.007	0.07	0.00179
4.	48.0	0.594	0.184	0.0250	0.25	0.00520	0.545	0.392	0.009	0.09	0.00187
5.	46.0	0.523	0.191	0.020	0.20	0.00421	0.462	0.328	0.008	0.08	0.00173
6.	49.0	0.648	0.264	0.024	0.24	0.00479	0.444	0.305	0.0085	0.085	0.00173
7.	38.0	0.530	0.292	0.015	0.15	0.00395	0.392	0.282	0.007	0.07	0.00184
8.	41.0	0.611	0.272	0.0205	0.205	0.005	0.292	0.184	0.007	0.07	0.00170
9.	45.5	0.516	0.184	0.02	0.2	0.00419	0.342	0.204	0.0085	0.085	0.00186
Mean		45.22				0.00460					0.00178
S.D.		± 4.783				± 0.000169					± 0.0000615

Note: Aliquot taken = 0.1 ml.
Water added = 0.9 ml.

APPENDIX TABLE 31. Three-day-old male

No. of Sample	Wt. of insect (mg)	Acid phosphatase activity					Alkaline phosphatase activity				
		Optical density of		Pi in the		Pi per mg. wt. of body (mg)	Optical density of		Pi in the		Pi per mg. wt. of body (mg)
		Expt.	control	aliquot (mg)	whole body (mg)		Exp.	Control	aliquot (mg)	whole body (mg)	
1.	47.0	0.432	0.184	0.015	0.150	0.00319	0.314	0.152	0.0095	0.095	0.00202
2.	45.0	0.594	0.342	0.0155	0.155	0.00344	0.516	0.342	0.010	0.10	0.00222
3.	42.0	0.392	0.184	0.013	0.13	0.00309	0.314	0.184	0.008	0.08	0.00190
4.	48.0	0.523	0.264	0.0165	0.165	0.00343	0.561	0.392	0.01	0.10	0.00218
5.	36.0	0.414	0.233	0.0115	0.115	0.00319	0.314	0.194	0.007	0.07	0.00194
6.	38.0	0.409	0.194	0.0130	0.13	0.00342	0.495	0.367	0.008	0.08	0.00210
7.	41.5	0.438	0.211	0.014	0.14	0.00337	0.362	0.226	0.0085	0.085	0.00204
8.	39.5	0.426	0.194	0.014	0.14	0.00354	0.577	0.432	0.008	0.08	0.00227
9.	33.0	0.488	0.332	0.0105	0.105	0.00454	0.426	0.314	0.0065	0.065	0.00196
Mean S.D.		41.11 ± 5.029				0.00331 ± 0.000155					0.00206 ± 0.000130

Note: Aliquot taken = 0.1 ml.
Water added = 0.9 ml.

APPENDIX TABLE 32. Four-day-old male

No. of sample.	Wt. of insect (mg)	Acid phosphatase activity					Alkaline phosphatase activity				
		Optical density of		Pi in the		Pi per mg. wt. of body (mg)	Optical density of		Pi in the		Pi per mg. wt. of body (mg)
		Exp.	control	aliquot (mg)	whole body (mg)		Exp.	control	aliquot (mg)	whole body (mg)	
1.	41.0	0.488	0.184	0.0185	0.185	0.00451	0.292	0.191	0.0065	0.065	0.00158
2.	51.0	0.594	0.264	0.020	0.20	0.00392	0.328	0.215	0.007	0.07	0.00137
3.	47.5	0.523	0.201	0.0196	0.195	0.00410	0.444	0.342	0.006	0.06	0.00126
4.	49.5	0.638	0.301	0.0210	0.210	0.00424	0.314	0.191	0.0075	0.075	0.00151
5.	39.0	0.516	0.252	0.0160	0.160	0.00410	0.495	0.414	0.0050	0.05	0.00128
6.	38.0	0.462	0.222	0.015	0.150	0.00394	0.292	0.211	0.005	0.05	0.00131
7.	31.5	0.444	0.215	0.0145	0.145	0.00460	0.530	0.438	0.005	0.05	0.00161
8.	43.0	0.577	0.292	0.017	0.170	0.00395	0.462	0.352	0.0065	0.065	0.00151
9.	45.0	0.620	0.292	0.020	0.20	0.00444	0.569	0.482	0.006	0.06	0.00133
Mean		42.83				0.00419					0.00141
S.D.		± 6.199				± 0.0002607					± 0.0001349

Note: Aliquot taken = 0.1 ml.
Water added = 0.9 ml.

APPENDIX TABLE 33. Five-day-old male

No. of sample.	Wt. of insect (mg)	Acid phosphatase activity					Alkaline phosphatase activity				
		Optical density of		Pi in the		Pi per mg. wt. of body (mg)	Optical density of		Pi in the		Pi per mg. wt. of body (mg)
		Exp.	Control	aliquot (mg)	whole body (mg)		Exp.	Control	aliquot (mg)	whole body (mg)	
1.	39.0	0.438	0.204	0.0145	0.145	0.00371	0.264	0.191	0.0045	0.045	0.00115
2.	42.0	0.569	0.292	0.017	0.17	0.00404	0.332	0.240	0.005	0.05	0.00119
3.	52.0	0.495	0.184	0.019	0.19	0.00365	0.314	0.204	0.0065	0.065	0.00125
4.	38.0	0.432	0.194	0.015	0.15	0.00395	0.426	0.342	0.005	0.05	0.00131
5.	39.0	0.509	0.272	0.0145	0.145	0.00371	0.462	0.357	0.005	0.05	0.00128
6.	41.0	0.475	0.215	0.016	0.16	0.00390	0.264	0.171	0.0055	0.055	0.00134
7.	44.0	0.475	0.184	0.018	0.18	0.00409	0.452	0.382	0.005	0.05	0.00113
8.	47.0	0.495	0.204	0.0175	0.175	0.00372	0.332	0.233	0.006	0.06	0.00127
9.	36.5	0.495	0.284	0.013	0.13	0.00356	0.444	0.372	0.0045	0.045	0.00123
Mean		42.05				0.00381					0.00123
S.D.		± 4.927				± 0.000184					±0.0000709

Note: Aliquot taken = 0.1 ml.
Water added = 0.9 ml.

APPENDIX TABLE 34. Six-day-old male

No. of sample.	Wt. of insect (mg)	Acid phosphatase activity					Alkaline phosphatase activity				
		Optical density of		Pi in the		Pi per mg. wt. of body (mg)	Optical density of		Pi in the		Pi per mg. wt. of body (mg)
		Exp.	control	aliquot (mg)	whole body (mg)		Exp.	control	aliquot (mg)	whole body (mg)	
1.	38.0	0.392	0.194	0.0125	0.125	0.00328	0.392	0.337	0.0035	0.035	0.00092
2.	36.5	0.444	0.191	0.0125	0.125	0.00352	0.414	0.332	0.0045	0.045	0.00126
3.	31.0	0.475	0.292	0.011	0.11	0.00354	0.264	0.201	0.004	0.04	0.00129
4.	42.0	0.496	0.276	0.013	0.13	0.00309	0.292	0.233	0.004	0.04	0.00095
5.	43.0	0.414	0.184	0.014	0.14	0.00325	0.444	0.372	0.0045	0.045	0.00104
6.	49.0	0.516	0.244	0.016	0.16	0.00326	0.392	0.310	0.005	0.05	0.00102
7.	46.0	0.538	0.292	0.0155	0.155	0.00336	0.264	0.181	0.005	0.05	0.00108
8.	42.0	0.523	0.280	0.0150	0.15	0.00357	0.432	0.362	0.004	0.04	0.00095
9.	40.0	0.414	0.191	0.0135	0.135	0.00337	0.292	0.222	0.0045	0.045	0.00112
Mean		40.72				0.00336					0.00107
S.D.		5.414				0.000160					0.000132

Note: Aliquot taken = 0.1 ml.
Water added = 0.9 ml.

APPENDIX TABLE 35. Seven-day-old male

No. of sample.	Wt. of insect (mg)	Acid phosphatase activity					Alkaline phosphatase activity				
		Optical density of		Pi in the		Pi per mg. wt. of body (mg)	Optical density of		Pi in the		Pi per mg. wt. of body (mg)
		Exp.	control	aliquot (mg)	whole body (mg)		Exp.	control	aliquot (mg)	whole body (mg)	
1.	47.0	0.450	0.233	0.0135	0.135	0.00287	0.432	0.409	0.0015	0.015	0.000319
2.	42.5	0.420	0.211	0.013	0.130	0.00305	0.233	0.191	0.002	0.02	0.000470
3.	40.5	0.495	0.292	0.012	0.120	0.00300	0.240	0.211	0.0015	0.015	0.000375
4.	37.0	0.460	0.260	0.012	0.120	0.00324	0.310	0.292	0.0015	0.015	0.000405
5.	36.0	0.382	0.204	0.0105	0.105	0.00291	0.332	0.305	0.0015	0.015	0.000416
6.	41.0	0.438	0.233	0.013	0.130	0.00317	0.240	0.211	0.002	0.02	0.000487
7.	48.0	0.414	0.184	0.014	0.140	0.00291	0.342	0.310	0.002	0.02	0.000416
8.	43.0	0.523	0.292	0.014	0.140	0.00325	0.211	0.181	0.002	0.02	0.000465
9.	39.0	0.516	0.323	0.0115	0.115	0.00294	0.292	0.272	0.0015	0.015	0.000384
Mean		41.50				0.00303					0.000415
S.D.		4.107				0.000143					0.000531

Note: Aliquot taken = 0.1 ml.
Water added = 0.9 ml.

APPENDIX TABLE 36. Eight-day-old male

No. of sample	Wt. of insect (mg)	Acid phosphatase activity					Alkaline phosphatase activity				
		Optical density of		Pi in the		Pi per mg. wt. of body (mg)	Optical density of		Pi in the		Pi per mg. wt. of body (mg)
		Expt	Control	aliquot (mg)	whole body (mg)		Exp.	control	aliquot (mg)	whole body (mg)	
1.	42.0	0.392	0.194	0.0125	0.125	0.00297	0.360	0.260	0.0065	0.065	0.00154
2.	46.5	0.611	0.414	0.0125	0.125	0.00268	0.475	0.362	0.007	0.07	0.00150
3.	39.0	0.594	0.409	0.011	0.11	0.00287	0.284	0.184	0.0065	0.065	0.00166
4.	43.5	0.523	0.332	0.0115	0.115	0.00264	0.495	0.392	0.006	0.06	0.00137
5.	39.5	0.362	0.171	0.0115	0.115	0.00291	0.444	0.342	0.0065	0.065	0.00164
6.	41.0	0.516	0.342	0.01	0.10	0.00243	0.337	0.240	0.006	0.06	0.00146
7.	36.0	0.523	0.362	0.01	0.10	0.00277	0.482	0.362	0.006	0.06	0.00166
8.	35.0	0.409	0.260	0.009	0.09	0.00257	0.372	0.292	0.005	0.05	0.00145
9.	38.0	0.561	0.372	0.011	0.11	0.00289	0.432	0.323	0.0065	0.065	0.00171
Mean		40.05				0.00274					0.00154
S.D.		± 3.635				± 0.0001803					± 0.000113

Note: Aliquot taken = 0.1 ml.
Water added = 0.9 ml.

APPENDIX TABLE 37. Nine-day-old male

No. of sample	Wt. of insect (mg)	Acid phosphatase activity					Alkaline phosphatase activity				
		Optical density of		Pi in the		Pi per mg. wt. of body (mg)	Optical density of		Pi in the		Pi per mg. wt. of body (mg)
		Expt.	control	aliquot (mg)	whole body (mg)		Exp.	control	aliquot (mg)	whole body (mg)	
1.	46.0	0.561	0.362	0.012	0.12	0.00260	0.450	0.342	0.0065	0.065	0.00141
2.	45.0	0.362	0.181	0.0111	0.11	0.00246	0.362	0.260	0.0060	0.06	0.00133
3.	37.0	0.488	0.342	0.009	0.09	0.00243	0.495	0.409	0.005	0.05	0.00135
4.	39.5	0.530	0.357	0.01	0.10	0.00253	0.310	0.233	0.0056	0.056	0.00141
5.	42.0	0.561	0.382	0.011	0.11	0.00261	0.438	0.342	0.006	0.06	0.00142
6.	46.0	0.569	0.382	0.012	0.12	0.00260	0.462	0.362	0.0065	0.065	0.00141
7.	44.0	0.362	0.184	0.011	0.11	0.00250	0.392	0.288	0.006	0.06	0.00136
8.	41.0	0.523	0.342	0.011	0.11	0.00268	0.432	0.352	0.0055	0.055	0.00134
9.	38.0	0.576	0.414	0.0095	0.095	0.00250	0.488	0.409	0.005	0.05	0.00131
Mean		42.05				0.00254					0.00137
S.D.		± 3.413				± 0.0000815					± 0.0000416

Note: Aliquot taken = 0.1 ml.
Water added = 0.9 ml.

APPENDIX TABLE 38. Ten-day-old male

No. of sample.	Wt. of insect (mg)	Acid phosphatase activity					Alkaline phosphatase activity				
		Optical density of		Pi in the		Pi per mg. wt. of body (mg)	Optical density of		Pi in the		Pi per mg. wt. of body (mg)
		Exp.	control	aliquot (mg)	whole body (mg)		Exp.	control	aliquot (mg)	whole body (mg)	
1.	48.0	0.342	0.191	0.0095	0.095	0.00197	0.226	0.181	0.0025	0.025	0.000520
2.	42.0	0.332	0.181	0.009	0.09	0.00214	0.252	0.211	0.002	0.02	0.000470
3.	45.0	0.462	0.362	0.008	0.08	0.00177	0.292	0.264	0.002	0.02	0.000440
4.	39.0	0.444	0.310	0.008	0.08	0.00205	0.332	0.280	0.002	0.02	0.000640
5.	37.5	0.332	0.211	0.007	0.07	0.00186	0.382	0.342	0.0025	0.025	0.000630
6.	32.0	0.342	0.252	0.007	0.07	0.00218	0.323	0.292	0.002	0.02	0.000460
7.	46.0	0.516	0.362	0.009	0.09	0.00195	0.382	0.342	0.0015	0.015	0.000540
8.	40.5	0.392	0.264	0.008	0.08	0.00236	0.414	0.372	0.0025	0.025	0.000617
9.	36.0	0.482	0.342	0.0085	0.085	0.00213	0.372	0.342	0.0025	0.025	0.000453
Mean		40.166				0.00201					0.000527
S.D.		± 5.155				± 0.0001824					± 0.000066

Note: Aliquot taken = 0.1 ml.
Water added = 0.9 ml.

APPENDIX TABLE 39. Eleven-day-old male

No. of sample	Wt. of insect (mg)	Acid phosphatase activity					Alkaline phosphatase activity				
		Optical density of		Pi in the		Pi per mg. wt. of body (mg)	Optical density of		Pi in the		Pi per mg. wt. of body (mg)
		Exp.	control	aliquot (mg)	whole body (mg)		Exp.	control	aliquot (mg)	whole body (mg)	
1.	37.0	0.516	0.372	0.0085	0.085	0.00229	0.222	0.194	0.0015	0.015	0.000405
2.	39.5	0.362	0.211	0.009	0.09	0.00227	0.382	0.342	0.002	0.02	0.000506
3.	32.0	0.342	0.233	0.007	0.07	0.00218	0.248	0.233	0.0015	0.015	0.000468
4.	49.0	0.392	0.194	0.012	0.12	0.00244	0.382	0.342	0.0025	0.025	0.000510
5.	42.0	0.495	0.342	0.009	0.09	0.00214	0.545	0.502	0.0025	0.025	0.000595
6.	46.0	0.414	0.222	0.0115	0.115	0.00250	0.392	0.342	0.0025	0.025	0.000543
7.	35.5	0.475	0.342	0.008	0.08	0.00225	0.314	0.284	0.002	0.02	0.000563
8.	32.0	0.475	0.352	0.0075	0.075	0.00234	0.414	0.392	0.0015	0.015	0.000483
9.	36.5	0.530	0.382	0.009	0.09	0.00246	0.516	0.475	0.0025	0.025	0.000684
Mean		38.83				0.00231					0.000528
S.D.		±5.90				±0.000126					±0.0000804

Note: Aliquot taken = 0.1 ml.
Water added = 0.9 ml.